STUDIES OF SOIL COPPER
IN RELATION TO
ITS AVAILABILITY TO PLANTS

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ABSTRACT

The adsorption of copper by various soil materials and intact soils was examined at equilibrium solution copper concentrations within the range found in natural soil solutions. The distribution of copper between solution and solid phases was measured by means of labelling with radioactive copper-64. In the majority of soils specific adsorption of copper by oxides and organic materials is considered likely to control solution copper concentrations. Although the adsorbed copper is strongly held, experiments have shown that a large proportion of it remains isotopically exchangeable and can still therefore be regarded as 'plant available'. Solution concentrations of copper were relatively unaffected by both the background concentration of major cations and by changes in pH within the ionic strength and pH range found in normal agricultural soils.

The relative abundance of different forms of copper in pasture and arable soils was compared by means of a fractionation scheme. The principle difference found was that the pasture soils contained higher concentrations of soluble copper complexes. A specific ion electrode was used successfully to differentiate between ionic and complexed copper in simple systems but suffered from interference by other ions when used with natural soil solutions.

Soil samples, both with and without added copper were incubated moist at a temperature of 20°C for a period of ten months. Duplicates were stored dry. Regular analysis showed that the amount of copper extractable with EDTA decreased with time, especially in the moist soils. Fixation of copper by soil oxides appeared to be

responsible. Additional changes were observed in both calcium chloride extractable and isotopically exchangeable copper which were not reflected by changes in the amount of copper extracted by EDTA.

Plant uptake studies with red clover under glass demonstrated that significant increases in plant copper content can be achieved by adding copper to a variety of soils. The size of the response varied considerably from soil to soil but no clear relationship was observed between the copper content of the controls or the response to copper additions and individual soil properties.

A second glasshouse trial, using barley, clover and ryegrass, indicated that there appears to be no advantage in using Cu-EDTA rather than $CuSO_4$ as a copper fertilizer. Clover responded to copper additions more readily than ryegrass and both plants responded far better when the copper fertilizer was mixed into the soil rather than applied to the surface.

The existence of interactions between copper and other trace elements was also demonstrated in pot trials. However, the nature and importance of such interactions seems to depend on the particular conditions under which an experiment is carried out.

DECLARATION

I hereby declare that this thesis was composed by myself and the work described was carried out entirely by myself except where indicated.

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ABBREVIATIONS

In some of the table headings it was necessary to use abbreviations which are not explained elsewhere, i.e.

soln.	≡	solution
conc.	≡	concentration
DM	≡	dry matter
incub.	≡	incubation
wks.	≡	weeks
OM.	≡	organic matter

1. INTRODUCTION

Copper is an essential element in the nutrition of animals and plants and thus is of considerable economic importance in agriculture. An inadequate supply causes low crop yields and poor performance of livestock in many parts of the world. However, although levels of copper in soil may be low in affected areas, the intake requirement of plants and animals is comparatively small. The principle reason for deficiencies is that only a fraction of the copper present in soil is in forms which can be readily taken up by the plant. The problem can be compounded by poor utilization of herbage copper by animals.

The history of known copper deficiency covers a period of only fifty years. Extreme cases were detected first because of severe effects on growth. As these were brought under control, larger numbers of cases were recognized because of increasing familiarity with the symptoms and a growing need to maximize grain yields. The problem is likely to increase in severity as well as extent, with deficiency symptoms becoming associated with soils in which copper availability is at present adequate. This is because higher grain yields and larger numbers of livestock to the hectare cause more copper to be removed from the soil while the input of copper, in the absence of supplementation, is decreasing. Whereas one application of farmyard manure supplied as much copper as a rotation of crops removed, the fertilizers now used contain only insignificant amounts (Williams et al, 1960). Soils with relatively low total copper levels, which are under cultivation, are subject to quite rapid depletion because copper levels in crops are often similar or higher.

The need for supplementation will thus become higher, while the rising costs of materials and labour will encourage greater efficiency in the treatment of deficiency, so that the minimum amount of copper can be applied with the minimum frequency. Treatment of animals and plants is possible, by diet supplements and spraying foliage respectively, but is not a complete answer. The other approach is to supplement the soil so that the supply of copper available to plants is sufficient for their nutrition and provides an adequate level in forage. In order to be able to do this with any efficiency far more information is required on the behaviour of copper in soils. At the present time the assessment and treatment of copper deficiency is made difficult by the lack of knowledge of the factors controlling the availability of native and added soil copper. Investigation is needed to provide information on the forms in which copper occurs in soils, their proportions and their role in supplying copper to plants. This is the purpose of this investigation.

2. LITERATURE REVIEW

2.1. Plants and copper

2.1.1. The history of copper deficiency

Observations of copper stimulating plant growth date from the earliest years of the century (Allison et al, 1927) and these were mostly associated with the use of the fungicide Bordeaux mixture. In the U.S.A., Lutman (1911) recorded that copper increased potato yield even in the absence of blight and observations of its effect led to the use of copper sulphate as a cure for dieback in citrus (Floyd, 1917).

The situations in which the effects of copper were first investigated involved severe copper deficiency, usually on reclaimed land, where complete crop failure was not uncommon. Organic, rather than mineral soils, were often involved. In Holland, Hudig and Meyer (1926) found that "reclamation disease" of oats was prevented for several years by a soil dressing of 50 kg $CuSO_4$ ha^{-1} but not by $MnSO_4$. Densch and Hunnius (1924), in Germany, found that in addition to raising yields the application of copper increased the uptake of copper by plants, suggesting a site of action within the plant. In the U.S.A., spectacular improvements were reported in various crops after adding copper to raw peat soils in Florida (Allison et al, 1927) and muck soils in New York (Felix, 1927). In both cases, it was suggested that the copper supplied was utilized in plant growth.

These reports led to attempts, using solution culture, to determine whether or not copper is essential for plant growth. The low

requirement for copper was not, however, appreciated at first and experimental plants often received an adequate amount from the water supply, perhaps from a copper still, or as impurities in the salts supplying other elements. Brenchley (1910, 1938) used a standard nutrient solution with varied copper additions and obtained no signs of deficiency. Sommer (1931), however, by using water redistilled from pyrex and purified salts, obtained sunflowers which failed to flower and poor growth of tomatoes and flax. Lipman and MacKinney (1931), using similar techniques, showed that no flax plant deprived of copper produced any seed capsule or seed and in a second experiment barley failed to produce seed in the absence of added copper.

These experiments showed that copper is necessary for the completion of the plant life cycle but the presence of copper in the seed prevented demonstration in this way that copper is essential for growth. This came in the following decade, with the isolation of enzymes, essential for metabolism, for the functioning of which copper is necessary.

In the field, further cases of deficiency were identified. Dunne (1938) concluded that the dying back of shoots of apple trees, which occurred on leached soils, was due to the lack of sufficient copper for normal growth. Riceman et al (1938) described symptoms of severe copper deficiency on very calcareous sands on the coast of South Australia, where the application of copper sulphate led to normal vegetative development and improved grain production. The findings of Hudig and Meyer (1926) in Holland led to the discovery of deficiency elsewhere in Europe. Large areas of peat and sandy soils in Denmark were found to be deficient (Steenbjerg and Boken, 1950) and

in Sweden and Finland grain yields were frequently increased by copper additions, chiefly to peaty soils (Lundblad et al, 1949; Jamalainen, 1950).

By half-way through the century, copper supplementation had made severe cases of deficiency rare but milder, less obvious cases were more frequent (Mulder, 1950). Applications of copper to soils showing no symptoms of deficiency resulted in increased plant yields and the need was expressed for a method of assessing plant-available copper in soils (Gilbert, 1948). Chemical tests were subsequently developed by Mitchell et al, (1956) and others.

In Britain milder forms of the problems experienced in Holland were found on similar soils in East Anglia. Deficiency symptoms were seen here in wheat on reclaimed peat and identified as such by Hudig in 1946 (Pizer et al, 1966). As well as on peat, deficiencies occur on leached glacial sands, shallow soils on the chalk of southern England and sandy loams in the North (Caldwell, 1971a). In 1952, wither-tip was spotted in south-east Scotland and six years later this was confirmed to be copper deficiency, when treatment of the affected oats enabled the plants to form ears. Many of the cases of deficiency subsequently seen were on soils on fluvioglacial sands derived from the Old Red Sandstone beds (Purves and Ragg, 1962).

2.1.2. Symptoms of deficiency

Observations of deficiency are most common in the cereals, through a combination of the economic importance of the crops and the stress placed on the soil-plant system. The symptoms vary accordingly to the degree of deficiency. In mild cases, there may be a slight retardation

of growth and fewer tillers but no definite symptoms until the ears emerge. These are smaller than normal and contain either small grains or are "blind", especially at the tip. Thus even a slight deficiency is of economic importance and it is likely that copper deficiency can also reduce yield without causing any visual symptoms.

In slightly more severe cases, the plant is obviously affected in the vegetative stage. Limpness of leaves is followed by necrosis of the young leaf tips. The necrosis can extend downwards along the leaf margins, possibly giving ring-like kinks in the leaves and spiral twisting and coiling of leaves of wheat and oats, which sometimes remain trapped in the apex of the subtending leaf. (Mulder, 1950; Reuther, 1957; Pizer et al, 1966; Greulach, 1973; Hewitt and Smith, 1975; E.S.C.A., 1975)

A form of copper deficiency peculiar to wheat is described as "melanism" (Hooper and Davies, 1968). In this condition patches of dark pigment appear, initially at the top internode, after the emergence of the ears. The plant becomes olive-green and grain yield is reduced.

Piper, (1942), using solution culture, obtained a more severe deficiency in oat plants. Growth of the tillers ceased and they dried to a bleached grey colour. The base of the plant, however, remained green and immature and there was considerable development of secondary tillers. The greeness persisted after comparable plants with an adequate copper supply had developed mature grain and become fully dried out but the copper-deficient plants eventually died.

Copper deficiency is not seen in pasture, because those plants at a disadvantage in a situation of low copper availability are

replaced in the sward by others. In the more intensive situation of an alfalfa crop, symptoms similar to those of cereals have been observed (Sauchelli, 1969) and Lolium subulatum, Trifolium subterraneum and Phalaris tuberosa exhibited corresponding symptoms when grown in a culture solution without added copper (Piper, 1942). Copper deficiency also reduces yields of maize, sugar cane, onions, peas and tomatoes (Sauchelli, 1969).

Symptoms of "dieback" or exanthma of citrus trees include the dying back of new growth, after stained spots appear on the bark from gum pockets underneath. Below the dead portions, multiple buds form in leaf axils and many side branches form. The internodes are short, causing the formation of "witch's brooms". The fruit also shows brown gum stains (Reuther, 1957; Sauchelli, 1969). Apple, plum, olive and pear trees exhibit similar symptoms (Hewitt and Smith, 1975) and saplings of spruce and Douglas fir become deformed if the supply of copper is inadequate (Strullu and Bonneau, 1968).

2.1.3. Variations in plant copper content between species

Symptoms of copper deficiency would be expected to reflect a lower copper concentration within the plant. However, the higher plant is such a complex and variable organism that even the "normal" copper content varies widely from species to species, from less than 2 to over 30 μg Cu g^{-1} dry matter (table 2.1.)

An important aspect of the variation is that the lower values tend to occur in pasture grasses. The levels may or may not be adequate for the plants; the former is likely in a mature pasture as a result of competition. Gladstones et al, (1975) found that plants

Table 2.1.

Copper levels in foliage

	Species	µg Cu g^{-1} in dry matter
	Anthoxanthum odoratum	2.1-7.7
Grasses	Vulpia sp.	7.2
	Bromus rigidus	6.7
	B. mollis	5.5
	Lolium rigidum	4.3
	Lolium perenne	5.0
	Lolium sp.	6.0, 2.5-3.3
	Poa pratensis	21.1, 20.4
	Agropyron repens	6.8
	Phleum pratense (timothy)	2.7-3.4, 5.5
	Cynosurus cristatus (crested dogstail)	2.9-3.7
	Dactylis glomerata (cocksfoot)	2.1-7.7
Clovers	Trifolium pratense (red clover)	17.5, 1.3-8.1
	Trifolium sp.	13.5, 18-22, 2-12
	Trifolium hirtum	8.9
Cereals	Hordeum sativum	3.7
	Avena sativa	3.8, 4.0
	Triticum aestivum	4.1
	Secale cereale	7.3
Herbs	Arctotheca calendula	11.5
	Erodium botrys	5.1
	"Buttercup"	16.6
	Taraxacum officianale	17.6
	Melampyrun silvaticum	31.7
	Oxalis acetosella	6.7
	Plantago sp.	15.9

Sources

Beeson et al, 1947
Erkama, 1947
Mitchell, 1948
Wells, 1956
Mitchell et al, 1957
Williams et al, 1960
Archer, 1968
Gladstones et al, 1975

adapted to deficient conditions contained relatively high copper levels but Beavington and Wright (1977) reported low herbage copper levels in tolerant species. Both authors agreed on the pastures being "poor" in an agricultural sense, so that "improvement" of the pasture, with the sowing of grasses of higher foliage yield, is likely. However, such varieties are insensitive to soil trace element supply (Alderman, 1968) and in conditions of low copper availability will often give livestock a diet inadequate in copper.

The high copper levels possible in clover (table 2.1.) are of interest, as an increased proportion of clover in a pasture would increase the overall herbage content of the element (Beavington and Wright, 1977; Gladstones et al, 1975). Unfortunately, the application of nitrogen fertilizers tends to eliminate clover from the sward (Alderman, 1968). The figures in table 2.1. show that almost any herb would similarly raise copper levels, as was observed by Archer (1968), but it is general practice to eliminate these also.

Jarvis and Jones (1979) suggested, on the basis of solution culture work, that the higher copper levels in clover shoots relative to ryegrass reflect the greater growth of clover roots and that slower shoot growth is also a factor. This needs investigation in soil-grown plants.

As well as variation between species, there are also differences in the copper contents of varieties of the same species (Caldwell, 1971a; Brown et al, 1972). Cannon et al, (1972) stressed the importance of discriminating between varieties when sampling corn and wheat, although Gladstones et al, (1975) found little difference among three varieties of Trifolium subterraneum and two of Avena sativa.

2.1.4. Variation in plant copper content with time

Where a range of values is given for herbage copper levels (table 2.1.), contributory causes are the season and stage of growth at which the sampling was carried out (Reith and Mitchell, 1964). Seasonal and yearly variation (Hemingway, 1962), reflecting the environmental factors acting on both plant and soil, will combine with variation within the plant to produce the changes observed.

Gladstones et al (1975) found that plants accumulated copper almost to maturity, but the concentration in the herbage declined with time. The latter trend, also reported by Thomas et al (1952) has been attributed (Voss and MacPherson, 1977) to the corresponding increase in the ratio of stem to leaf. Beeson and MacDonald (1951) also found, in Timothy, a decrease in concentration with growth, as did Wells (1956) in Anthoxanthum odoratum, although in the latter case the copper content was fairly constant until it fell with seed formation.

Legumes behave differently in that there is a peak in copper concentration at an intermediate "midbloom" stage of growth (Beeson and MacDonald, 1951). Gladstones et al (1975) reported that lupins ceased accumulating copper before maturity and observed that the concentration difference between legumes and grasses increased late in the growing season.

Lane and Fleming (1967) examined copper levels in ryegrass both at different stages of growth and on regrowth from the same plants. They recorded no variation in either case, but the plants were grown under glass. Hemingway (1962) had previously found that copper levels in both grass and clover rose over a series of harvests.

2.1.5. The distribution of copper within the plant

The values given in table 2.1. are from analysis of foliage. Root analysis is generally avoided because of the difficulties in separating roots from soil. A number of workers have, however, compared copper concentrations in roots and shoots of the same plant and found higher concentrations in the former in a variety of species (Ishizuka, 1942; Bhatti and Sarwar, 1977; Jarvis, 1978; Graves, 1978). Root concentrations may be up to twelve times the shoot values (Dykeman and Desousa, 1966). The cause of the difference is in the roots. Smith et al (1949) grafted Valencia orange to six stocks and showed that the roots controlled the leaf copper concentration.

This trend of lower copper levels in the shoots is of importance, since it is the shoots which bear the grain or provide foliage and, unless plants are sprayed with the element, copper must reach the shoots via the roots. If only a fraction of copper taken up by the roots reaches the shoots this could be an important factor in causing copper deficiency.

There is a growing amount of evidence, however, that the relationship between root and shoot concentrations is not unaffected by the amount of copper involved, that the "barrier" between roots and shoots is a protective mechanism against high copper levels. Struckmeyer et al (1969) and Graves (1978) refer to "accumulation" of copper in roots, suggesting that the proportion reaching the shoots is not fixed. Values are provided by Jarvis (1978). The addition of 24 kg Cu ha^{-1} soil increased retention by ryegrass roots from 40.5% to 60.3%, raising the concentration in roots by 102% and that in shoots by 42%. In an extreme case, 92.5% retention by roots has been recorded, with a

root concentration of 4040 $\mu g\ g^{-1}$ (Dykeman and Desousa, 1966).

It is possible that all the examples quoted are, in fact, demonstrating the response of plants to excessive concentrations of copper in solution, a situation that is especially likely in solution culture (Bennett, 1974). Although Jarvis (1978) found that the copper accumulated in roots was not available for transport to the shoots, it is stated in a later paper (Jarvis and Jones, 1979) that the proportion of the copper in the roots which could not be translocated decreased as the total content increased. This suggests that the capacity of the roots for retaining copper is finite.

Nielsen (1976a), concerned about shoot analysis providing a poor estimate of plant copper intake, investigated the situation in experiments involving water and pot culture of barley. He concluded that the ratio of "uptake" by the top to uptake by the whole plant remained unaffected by variation in solution copper concentration between 0.08 and 1.6μM, i.e. 0.005 - 0.096 $\mu g\ ml^{-1}$. This covers the range of copper concentrations to which a root is normally exposed (table 2.4.). It appears that, under normal conditions, a fixed proportion of copper entering the plant reaches the shoots. When the plant's uptake of copper is reduced below a certain level, the accumulation in roots is decreased (Graves, 1978). Thus copper deficiency in plants would not seem to be due to poor distribution within the plant.

Within the shoots, the leaves contain more copper than the stems and petioles (Beeson and MacDonald, 1951), with the highest concentrations in chloroplasts. The mobility of copper depends on species, age of plant and season (Sauchelli, 1969; Hewitt and Smith, 1975). Copper is normally retranslocated from vegetative tissues to forming

grain. This transfer is usually independant of copper levels (Reith and Mitchell, 1964) but may fail to take place under deficiency conditions (Hill et al, 1978).

2.1.6. Uptake of copper by plants

The copper content of a seed is small relative to that of a mature plant, in the order of 0.15 - 1.5 µg in cereals (Hewitt and Smith, 1975). Airborne supplies are insignificant (Archer, 1968), so that copper coming from the soil must form the bulk of the supply.

Little is certain about the mechanism of uptake of copper. Micronutrient uptake is not appreciably antagonised by relatively large changes in much higher concentrations of macronutrients. Uptake of copper is also against a concentration gradient. Cell sap has been analysed at 0.18 - 0.60 µg ml^{-1} with respect to copper in plants grown in nutrient solution containing 0.06 µg Cu ml^{-1} (Hewitt and Smith, 1975). Thus, uptake of copper appears to be an active process involving specific sites. The description by Jenny and Grossenbacher (1963) of the "mucigel" filling the space between cell wall and soil particles suggests that there is no lack of contact between soil and root surfaces. The mechanism of uptake by plants does not appear to be a limiting factor in the passage of copper along the soil-plant-animal chain, yet the plant does not always receive an adequate supply of copper.

2.1.7. The role of copper in higher plants

Copper is an essential element, yet the amounts required are small. This is because the role of copper is that of activation of

certain enzyme molecules. Associated with one functioning molecule of these enzymes will be between one and eight copper atoms, possibly combined in different ways. In some cases, the copper atom performs its role by changing valency in order to transport electrons, thus mediating in oxidation. However, other copper atoms function apparently without a change of valency. Both forms can occur in the same enzyme molecule. Enzymes of higher plants have diverse properties and a wide range of substrates, including carbohydrates, amines, phenols and ascorbate (table 2.2.).

In addition, several stages of nitrogen metabolism involve copper, for example, the conversion of NO_3 to NO_2, and a role of copper has been suggested in cell wall formation (Hewitt and Smith, 1975).

2.1.8. Toxicity

Plants are adversely affected by high levels of copper as well as by deficiency. The majority of descriptions of toxicity symptoms are from plants in solution culture (Struckmeyer et al, 1969; Bennett, 1974) where there is no material capable of adsorbing the copper, or from pot experiments to which copper has been recently added (Drouineau and Mazoyer, 1962; Purves, 1968). Nielsen (1976b) found that two months was required for the equilibration of treatment copper with soil in pots. In the field also, adsorption will render excess dissolved copper harmless, up to the capacity of the soil, in a comparable period of time. The only point for concern is that the adsorption capacity is finite and saturation may be imminent where coarse-textured soils have a history of copper applications (Reuther

Table 2.2.

The roles of copper in higher plants

Protein	Copper content	Valency change	Role
Plastocyanin	1 atom/molecule, sometimes 2	Yes	An electron transport protein linking photosystems I & II in photosynthesis
Cytochrome a-a_3 complex	1 or 2 atoms link the cytochromes	Yes	Part of respiration - the copper functions as an intermediate electron transport link
Ascorbic acid oxidase	8 atoms/molecule	Yes	Catalyses the direct oxidation of ascorbic acid
Catecholase/ polyphenol oxidase	4 Cu^{+}/molecule		
Tyrosinase	1 Cu^{+}/molecule	No	Causes melanin formation on injury
Laccase		Yes	Oxidises phenolic compounds
Diamine oxidase	1 Cu^{+}/molecule	No	
Umecyanin	1 atom/molecule	Yes	Oxidised by O_2 in the presence of laccase
Stellacyanin			
Ribulose-diphosphate carboxylase	1 tightly bound Cu^{2+}/molecule		
Superoxide dismutase	May contain Fe, Mn or 2Cu + 2Zn		Possibly protects macro-molecules against free radical oxidations

Sources : Hewitt and Smith, 1975; Sauchelli, 1969.

and Smith, 1953).

Toxicity may be associated with levels of copper in culture solution from 0.04 $\mu g\ ml^{-1}$ upwards (Bennett, 1974) or EDTA-extractable levels in soil in pots above 30 $\mu g\ Cu\ g^{-1}$ (Purves, 1968). Under these conditions, roots are shorter and thicker with shortened, closer laterals; these are similar symptoms to those associated with chlorosis. Leaves have an abnormal cellular structure (Struckmeyer et al, 1969) and a modified nutrient content. High copper levels can cause iron deficiency (Reuther and Smith, 1953), suggesting that the symptoms described in roots are those of chlorosis.

2.2. Animals and copper

2.2.1. History of deficiency

The first naturally occurring copper deficiency of animals was reported in Florida and called "salt sickness" (Neal et al, 1931). A drench of iron plus copper formed an effective treatment but iron was inadequate alone. Sjollema (1933) described a disease of cattle occurring in Holland in conjunction with copper deficient vegetation. This "Lecksucht" (parched disease) was cured by copper, which the author suggested had a physiological role.

In Australia, Bennetts and Chapman (1937) showed that the disease enzootic ataxia, of new born and young lambs, was prevented by giving copper supplements to pregnant ewes. Bennetts and Beck (1942) concluded that copper deficiency was the cause of ataxia and Innes and Shearer (1940) showed swayback, found in Britain, to be pathologically similar.

With some occurrences of deficiency, pasture copper levels suggested a dietary deficiency of copper (Barlow and Purves, 1960) whereas in other cases, both of swayback (Innes and Shearer, 1940) and copper deficiency in cattle (Allcroft and Parker, 1949; Jamieson and Allcroft, 1950), the pasture contained normal levels of copper. The trace elements molybdenum and sulphur were suspected of inducing copper deficiency by reducing its availability to animals (Dick and Bull, 1945; Dick, 1953a and b). Matrone (1970) concluded that copper deficiency could be induced in the ruminant by the formation, involving sulphate, of an inert Cu-Mo complex. The importance of the interaction was indicated when Suttle (1975) found that variations

in dietary S and Mo within the normal range for herbage affect the Cu and Mo metabolism of the grazing animal.

2.2.2. The utilization of copper by animals

The role of copper in animals is similar to that in plants, with some copper-requiring enzyme systems common to both. Additional functions include enabling the utilization of iron for the formation of haemoglobin (Sauchelli, 1969) and a role in the formation of the structural materials elastin and collagen (Mills, 1975).

The requirement for copper in animals, as in plants, is low but the efficiency of utilization of herbage copper is also low, being less than 10% for ruminants (Suttle, 1979). Thus, although a plant may contain sufficient copper for its own nutrition, this need not supply sufficient copper to the animal which consumes it. Minimum dietary requirements of 5 $\mu g\, g^{-1}$ for sheep and 10 $\mu g\, g^{-1}$ for cattle have been suggested (A.R.C., 1965). The highest requirement for copper is in the pregnant female. This is because the liver of the foetus accumulates copper, in order that the young animal may survive while receiving only the negligible supply of copper present in the mother's milk (Bennetts and Chapman, 1937). Thus copper deficiency is most likely in either the mother or the young.

2.2.3. Symptoms of deficiency

Swayback or neonatal ataxia occurs in many parts of Britain. It is a spastic paralysis of the limbs, usually fatal, which occurs in lambs of ewes receiving an inadequate supply of copper (Innes and Shearer, 1940). The symptoms usually occur at birth but can be

delayed for up to a year (Voss and MacPherson, 1977).

In copper-deficient areas of Australia, enzootic ataxia causes a similar inco-ordination and death in lambs, or at best retarded growth, which usually occurs between the first and second months (Bennetts and Chapman, 1937; Sauchelli, 1969). In some cases, adult sheep are also affected by copper deficiency, having "steely wool" which is straight and hairy, without elasticity, and grows slowly (Bennetts and Beck, 1942). In Britain, similar but less severe symptoms have been observed where the improvement of hill pasture has enhanced herbage Mo and S contents (Whitelaw et al, 1977, 1979). Affected lambs had fragile bones; they also weighed less and had poorer fleeces than twins receiving copper supplements.

Calves rarely suffer as severely as lambs born in the same area (Sauchelli, 1969) but a cow deficient in copper can give birth to weak stunted calves with bone deformities (Voss and MacPherson, 1977) and the "Wobbles" affects foals and calves in Australia and the U.S.A. (Sauchelli, 1969). In the absence of copper, cartilage may be formed without the simultaneous deposition of minerals in the matrix (Voss and MacPherson, 1977) and the "falling disease" of dairy cattle in Australia is due to the sudden failure of weakened heart muscle (Sauchelli, 1969).

In less severe cases, symptoms of deficiency in cattle occur in the first growing season. The coat becomes rougher, black hair may become brown through loss of pigment and characteristic unaffected areas ("spectacles") are seen around the eyes. Associated with coat condition is poor growth, anaemia and often profuse scouring (E.S.C.A., 1975).

2.2.4. Toxicity

Most mature animals are able to excrete copper and thus avoid poisoning from high levels of the element . The sheep is an exception to the rule and thus requires a more cautious approach to the supplementation of its diet. Young animals are at greater risk. Calves retain copper in the liver and toxicity has occurred in lambs on diets containing less than 20 μg Cu g^{-1} (Hill, 1975).

2.3. The origin of soil copper

2.3.1. The source of soil copper

By far the major source of copper present in the soil is the parent rock from which the soil mineral fraction was derived. Copper is not uniformly distributed among rocks of different types, as figures for the Skaergaard igneous intrusion indicate. The magma contains 130 μg Cu g^{-1} but minerals formed from it contain from 20 to 600 μg Cu g^{-1} (Mitchell, 1964).

The incorporation of copper in a forming mineral depends primarily on the ionic radius, charge and electronegativity of the ion being appropriate (Jenkins, 1968). Copper has a high ionisation potential and high electronegativity with the effect that it substitutes only occasionally in silicate structures (Krauskopf, 1972). Thus acid granophyre of the Skaergaard intrusion, which is similar to granite, contains only 20 μg Cu g^{-1} (Mitchell, 1964). Concentrations are far higher in basic ferromagnesian rocks; the ferrogabbro of the Skaergaard intrusion, for example, contains 600 μg Cu g^{-1}. For incorporation of copper in a potential host mineral to occur, it must be present in the magma when the mineral is forming. Sulfides of copper are frequently formed, removing most of the copper before sodium plagioclase crystallises out (Jenkins, 1968). The relative lateness of crystallisation of acidic rocks further lessens their chance of containing copper (Mitchell, 1964).

Eventually, igneous rock is weathered away, providing materials from which sedimentary rocks can be formed. The bulk of copper in igneous rocks is in the more easily weathered constituents (Mitchell, 1964); thus the element becomes available for incorporation into

secondary minerals. Of sedimentary rocks, 80% are shales (Mitchell, 1964). During the formation of the latter, the silicates reorganise to form hydrolysed aluminosilicates with a layered structure (Hodgson, 1963). Formation of these clay minerals in a medium containing trace elements from weathered rocks allows adsorption and a degree of substitution of copper in the clay lattice. Mitchell (1964) gives a range of copper contents of 20 - 200 $\mu g\ g^{-1}$.

Sandstones are next in abundance amongst sedimentary rocks. These are composed almost entirely of minerals that weather with difficulty (Mitchell, 1964), which also do not contain high amounts of copper. Carbonate rocks, that is limestones and dolomites, are low in copper but weathering concentrates the less soluble residue, which is comparatively copper-rich (Hodgson, 1963). Metamorphism of sedimentary rocks alters clay minerals into forms more closely related to those of igneous rocks (Mitchell, 1964).

2.3.2. The fate of copper during soil formation

Physical and chemical weathering of the parent rock lead to the formation of the soil mineral component. Copper is released primarily as Cu^{2+} (Krauskopf, 1972) and has two possible fates. It can be lost from the soil by leaching or retained by the mineral fraction.

Levels of copper found in soil vary between about 1 and 200 $\mu g\ g^{-1}$, although most values fall between 3 and 60 $\mu g\ g^{-1}$ (Reuther, 1957). It has been stated (Hodgson, 1963; Ure *et al*, 1969) that copper levels are generally lower in soils than in the parent material, the latter workers finding that the mean soil content was less than half the average crustal abundance. This suggests that there is a substantial loss of copper by leaching during soil formation. However, figures

published by Jenkins (1968) indicate that a trend based on an average can be misleading. The mode of the copper contents of 127 top soils of Snowdonia was 24 $\mu g\ g^{-1}$. Values for basic igneous rocks had a mode of 42 $\mu g\ g^{-1}$ (much higher than the soils) but the figure was 10 $\mu g\ g^{-1}$ for shales and even lower for rhyolitic rocks. The relationship between rock and soil values depends on the solubility of the components containing copper.

It is possible that, during the initial stages of the formation of a soil, especially from igneous material, there may be a considerable loss of copper but once there is an established body of particulate matter, adsorption prevents leaching since, of the micronutrient elements, copper is probably the one most strongly adsorbed by soil surfaces (Krauskopf, 1972). Certainly, copper has been shown not to be leached from mature soils. Jones and Belling (1967), working with radioactive copper, found that, after the equivalent of 457 mm of rainfall, there was no movement at all of 7.85 kg $CuSO_4 . 5H_2O\ ha^{-1}$ down columns of peat, sand and terra rossa soils. A deep sand containing only 0.18% organic matter allowed 20 - 30 mm movement. In a similar experiment, Korkman and Virta (1979) recovered the bulk of the copper added from the uppermost 25 mm of various soils after the equivalent of 1035 mm rainfall. However, in podsols loss of copper is more likely. Soluble organic matter, such as fulvic acids, which is leached down the soil profile, may carry with it copper and other metal ions in the form of soluble complexes (Ong and Bisque, 1968; Schnitzer, 1969).

2.4. Forms of copper in soils

2.4.1. Methods of investigation

Two approaches are possible. Several workers, for example Martens (1968) and Zietecta (1975), have calculated correlations between the amount of copper removed by an extractant and the relative abundance of particular soil components in different soils. This enables a suitable extractant to be chosen for removing a precise fraction of soil copper, that bonded in a particular manner or associated with a certain material. Such studies lead to a fractionation scheme (Grimme, 1967a and b; McLaren and Crawford, 1973a; Shuman, 1979), which consists of extracting a soil sequentially with progressively harsher extractants. Each extractant removes a known form of copper, enabling a picture to be built up of the distribution of copper within the soil.

A complementary technique is the study of the interaction between copper and soil materials, usually by determination of the amount of copper adsorbed or retained and the strength with which it is held. Copper retention can be investigated in complete soils but interpreting the contribution of different components is then limited to statistical comparison with soil properties (e.g. Lal et al, 1971). More information is obtained when the retention experiments are carried out with isolated soil components.

Both fractionation and retention studies have provided a body of information on the interactions of different soil materials with copper. However, the latter have generally been carried out with much higher levels of copper than are involved in the reactions naturally

occurring in soil. Three reasons are likely : -

1) In some cases, the unit of copper addition has been the cation exchange capacity of the material (e.g. Misra and Tiwari, 1966) and many workers have commenced with a material saturated with copper and then studied its removal. At this scale, the amount of copper adsorbed by the same material in natural soils is insignificant.

2) Those who recognised this problem and made smaller copper additions used levels which, although chemically low, were still high in biological terms. For example Kisk and Hassan (1973), in their adsorption studies, used a solution 0.0015N with respect to copper. This is, in fact, a concentration of 48 μg Cu ml^{-1}.

3) The copper concentration in a solution in equilibrium with a material has rarely been determined. This cannot be explained completely by the technical difficulties involved since, in many cases, concentrations would have been considerably higher than the soil solution copper levels determined by Hodgson et al (1965) and others since.

A second failing, mainly of older work, is not to represent in studies of retention the quantity relationship between macronutrients and copper that exists in normal soils. In order to represent the extent to which copper is adsorbed in such soils, an adsorption system must include an appropriate level of macronutrient cations, such as calcium. In most soils the ratio of calcium to copper is in the order of 100,000 : 1. A significant amount of adsorption of copper under these conditions implies the existence of sites which are highly specific for copper and possibly other trace elements. Adsorption which takes place in the presence of a high concentration of calcium

or other macronutrient cations is referred to as specific adsorption.

McLaren and Crawford (1973b) obtained specific adsorption capacities of 340 - 5,780 μg Cu g^{-1} for soils with total copper contents of 4.4 - 63.5 μg g^{-1}, which indicate that, under natural conditions, the specific sites are far from saturated. The authors suggested that, at levels of copper found in the soil, adsorption is completely or predominantly by specific sites. If, however, a large addition of copper is made to the soil, the specific sites will be saturated and the majority of it will compete, with any other ions present, for cation exchange sites. The predominant reaction studied, in this case, will be normal cation exchange and not specific adsorption. Since different reactions are involved, the extrapolation of results from high down to low solution concentrations will not be valid. An indication that this is so is given by published results. Adsorption isotherms obtained at relatively high copper concentrations form a curve that fits the Freundlich or Langmuir equations. The part of the graph closest to the origin - relating to conditions at the lowest concentrations - tends to be a straight line (McKenzie, 1967; McLaren and Crawford, 1973b; Cavallaro and McBride, 1978).

2.4.2. The association of copper with soil particles of different sizes

The soil components of larger particle size, the sand and silt fractions, play only a minor role in copper retention because of the relatively small surface area for adsorption. Plessis and Burger (1971), obtained significant adsorption by silt but the copper was only weakly held. Because of this the adsorption is of little significance. Where stronger adsorption is associated with these

fractions, it is often attributable to oxide coatings of the particles (Le Riche and Weir, 1963).

The proportion of the structural copper of the soil which is incorporated in the larger particles is variable. In older soils it will be small because the more resistant minerals tend to have a low content of micronutrients (Mitchell, 1964).

The materials to which most attention has been paid because of the degree of retention found, that is clay minerals, organic matter and free oxides, all include particles in the colloidal state. Adsorption by colloidal particles is likely to be high because of their large surface area, several thousand times more than the same mass of particles of the sand fraction.

2.4.3. The association of copper with organic matter

Rademacher (1936) suggested that copper was fixed by soil organic matter and the association was demonstrated in the soil by Kline and Rust (1966). They irradiated soil samples to which copper had been added and then equilibrated them with a copper sulphate solution. The diffusible fraction of soil copper thus determined was very similar to the fraction associated with organic matter, as determined by extraction with 0.5M NaOH. Nafady and Lamm (1972) obtained greater adsorption of copper by organic matter than by clays and according to the fractionation results of McLaren and Crawford (1973a) an average of 28% of soil copper is associated with organic matter. In addition, Sethi and Chopra (1976) observed that adsorption of copper by soil increased, and desorption decreased, as organic matter was added.

Organic matter is composed of compounds with a wide range of molecular weights and reactive groupings. Hodgson (1963) listed three classes of organic matter with which copper combines : -

1) Compounds of high molecular weight which immobilise the elements.

2) Short-chain organic acids and bases which promote solubility and movement.

3) Soluble complexing agents which form insoluble salts with heavy metals (and possibly soluble complexes when metal levels are lower).

A simpler division of soil organic matter is into that which forms part of the solid phase and that which, in natural soils, is in solution.

2.4.3.1. Solid-phase organic matter

Insoluble organic matter is conventionally divided into the material extracted by alkali and the residue, humin. The alkali extract is further divided, according to the precipitation by acid, into humic acid and fulvic acid (Buckman and Brady, 1960).

It is not possible to precisely define these fractions in terms of molecular weight because of the part played by the reactive groupings in determining the properties of any compound. The figures that are available are an average molecular weight of fulvic acid of 670 (Schnitzer, 1969) and a peak in abundance of humic compounds extracted with NaOH at a molecular weight of about 100,000 (Hayes and Swift, 1978). The latter authors also gave an extreme range of 2,000 - 1,500,000 for humic acid.

There does not appear to be any fundamental difference between

the various fractions of organic matter in terms of copper adsorption. Lewis and Broadbent (1961b) found that the proportions of different types of active sites were similar in both organic matter and its alkali extract, although, if equal weights were compared, the latter contained a greater number of sites.

In some soils up to half the copper is associated with the humic fraction (Manskaya and Drozdora, 1959; Mirchev, 1978) and there has been a considerable bias, in the number of investigations carried out, towards this fraction. However, it is not necessarily the most important with regard to adsorption of copper. Singh (1971) carried out experiments in which soil organic matter was replaced by increasing amounts of humic acid. Not only did the soils with added humic acid retain less copper than those with the original organic fraction but also the humic acid "fixed" a smaller proportion of the adsorbed copper than did the complete organic matter. This cannot be explained as a result of the alteration of humic acid by extraction, since this tends to increase the retentive capacity (Davies et al, 1969).

Adsorption of copper by the fulvic fraction may account for much of the difference described above. Bloomfield and Saunders (1977) found that, on a weight-for-weight basis, the dialysable fraction of organic matter extracts retained more copper than the colloidal fraction, suggesting that the low molecular weight fulvic fraction is of importance in copper retention.

A mixture of copper sulphate and fulvic acid adsorbed light at different wavelengths to the components, indicating to Broadbent and Ott (1957) that interaction had occurred. Delas (1967) found that, over a period of 50 years, stable complexes, some of which were

electronegative, had formed between copper and both humic and fulvic acids. Bondarenko (1972) differentiated between complexes of copper with humic and fulvic acids. The former were more stable at "neutral and weakly alkaline pH values" whereas copper-fulvic acid complexes were more stable under weakly acid conditions. A high stability is generally associated with copper-humic acid complexes (Manskaya and Drozdora, 1959; Ennis and Brogan, 1962; Petruzelli and Guidi, 1976).

2.4.3.2. Organic matter in solution

The first suggestion that copper in soil solution is not wholly ionic came when Beckwith (1958) speculated, from the lack of effect of the complexing agent EDTA on the relationship between transpiration and copper uptake by oats, that, in the fen soil used, plant uptake of copper was almost completely dependent on small amounts of soluble complexing agents increasing the amount of metal in solution.

Tobia and Hanna (1961) determined that 23% of the copper in a displaced soil solution was in the form of complexes stable enough to pass through a cation exchange column unchanged. However, since considerable additions of copper were made to the soil before displacement, it is unlikely that the solution obtained represents that of natural soils.

Hodgson et al (1965, 1966) allowed soils to equilibrate with added moisture before displacing the soil solution and investigated the extent of complexing by measuring the distribution of metal between aqueous and organic phases of a 2-phase system. In ten mineral soils between 76 and 99.5% of the copper in solution was complexed, while in 20 calcareous soils the minimum was 98%.

Mercer and Richmond (1969, 1970, 1971), using gel filtration,

identified four fractions of organic matter associated with copper in soil solution. These had molecular weights of below 200, between 300 and 400, between 1,400 and 1,500 and over 5,000. The material involved appears to be fulvic acid, with humic acid perhaps forming the fourth fraction. This last association is stable and incorporates up to half the copper in soil solution from copper deficient soils. This corresponds with the finding of Geering and Hodgson (1969) that the non-dialysiable fraction of organic matter in soil solution was more effective in complexing copper than the dialysiable fraction.

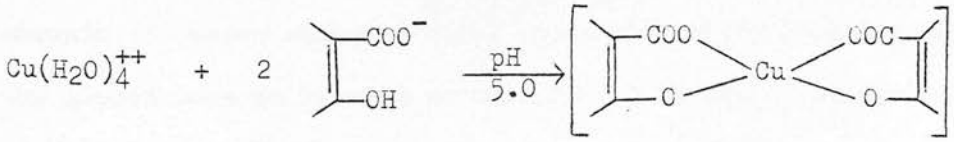
2.4.3.3. The nature of the organic sites adsorbing copper

Although the results of Demumbrum and Jackson (1956a) were interpreted as indicating specific adsorption of copper, the observed increase in exchange capacity could have been due to the acetate added (Bingham et al, 1964; McBride and Mortland, 1974). However, Broadbent (1957) determined, by elution, that some functional groups of organic matter combine with copper but not with calcium. Specific adsorption is also indicated by the report of Nafady and Lamm (1972) that the addition of organic matter to soil decreased the activity ratio, a(Cu)/a(Ca+Mg), and McLaren and Crawford (1973b) showed by regression analysis that organic matter, with free Mn oxides, contributed most to specific adsorption of copper by 23 soils.

The complexity of organic matter makes it unlikely that only one kind of site is responsible for copper retention. Davies et al,(1969) allowed copper-humic acid complexes to equilibrate several times with N HCl. This removed "virtually all" the copper but the strength of binding increased as the copper content decreased, in a way which suggested that copper is held by more than one type of site even when

present at low levels. Petruzzelli and Guidi (1976) also proposed different kinds of Cu - OM linkages. They found that, in a Neubauer seedling test, plants took up copper only from weaker sorption sites on humic substances and results of gel chromatography suggested that the constituents of higher molecular weight hold copper in a state of low availability to plants.

Eriksson (1949) predicted, from stability products and complex constants of different compounds, that organic matter would bind copper via groups containing oxygen, nitrogen or sulphur atoms. Demumbrum and Jackson (1956b) obtained evidence from optical adsorption studies that double bonds were involved. Goodman and Cheshire (1973) found that, in a copper-treated alkali peat extract, some of the copper was complexed by the nitrogen of porphyrin groups. Sulfhydryl groups also are effective in copper retention (Grewal et al, 1969). In the majority of cases, however, the groupings implicated in copper retention have been acidic. Lewis and Broadbent (1961a) concluded that retention of copper by organic matter involves carboxyl groups and phenols of varying acidity. Copper was adsorbed by the former as $CuOH^+$ but the phenols tended to complex the divalent cupric ion. These results were confirmed when other workers obtained a significant reduction in copper retention by blocking either acidic carboxyl or phenolic hydroxyl groups, both in humic and fulvic extracts and there was some retention by less acidic carboxyl groups (Schnitzer and Skinner, 1965; Schnitzer, 1969). In the latter paper appeared the following mechanism for the reaction between Cu^{2+} and fulvic acid :



The blocking experiment was repeated by Davies et al (1969) and the reduction in retention quantified at half the capacity of humic acid to firmly bind copper.

According to Ong and Bisque (1968), the reaction between metal ions and soluble soil organic matter is adsorption, similar to that occurring on the solid phase, rather than true complexing. Mercer and Richmond (1970) subsequently demonstrated, using chelating resin, that over half the copper associated with organic matter in two samples of soil solution was tightly bound.

2.4.3.4. Native and added copper

Experimental results quoted above suggest that, at lower solution copper levels, copper is tightly held by organic sites. Field results, however, show that, after the supplementation of soil with copper, higher levels of copper are available to plants for at least 18 years (Reith, 1975). This suggests that copper added recently to soil is combined with organic matter in a different manner to that which has been in combination longer, for example, the copper associated with the remains of living organisms. In support of this idea, Martens (1976) found that applied copper which was weakly bound to organic matter and oxide surfaces supplied the major part of the copper available to plants. Also, while studying organic matter in soil solution, Mercer and Richmond (1970) found that the distribution of added copper among materials of different molecular weights was dissimilar to that of the original copper. It appears that a clear distinction should be made between studies of the fate of significant amounts of copper added to soil, a category which includes much of the quoted work on organic matter, and examinations of the distribution

of copper in unsupplemented soil.

2.4.4. The association of copper with clay minerals

Clay minerals, such as illite, montmorillonite and kaolinite, contribute to the colloidal fraction of most soils and adsorb copper (Mitchell, 1964) because isomorphous substitution and exposed crystal edges cause clay particles to have an overall negative charge and thus attract cations. Interest in copper-clay interactions was increased by results suggesting that the exchange capacity of clays for copper was higher than for macronutrient cations such as Ca^{2+} and NH_4^+. Bower and Truog (1941) believed this to be due to adsorption of weak base-forming polyvalent cations, such as Cu^{2+}, as "$(HO - Cu)_{2x}$ - clay", rather than "Cu_x - clay", and thought reduced availability of copper to plants likely.

Antipov-Karataev (1947) obtained stronger sorption of copper than calcium and magnesium by montmorillonite, although the trend was reversed with "non-montmorillonitic" clays. Similarly, Menzel and Jackson (1950) obtained, in the presence of $(ClO_4)^+$, excess retention of copper by montmorillonite but with kaolinite more potassium was released than copper adsorbed.

The principle evidence of specific adsorption of copper by clays was provided by DeMumbrum and Jackson (1956a). Montmorillonite accumulated copper from a dilute solution to 10% of the exchange capacity, in spite of this being saturated with calcium. The calcium was, however, supplied in the form of $Ca(OAc)_2$: Bingham et al (1964) have determined that, in acetate systems, retention of copper exceeds the cation exchange capacity and McBride and Mortland (1974) attributed this to the formation of $Cu(OAc)_2$.

In a second paper, DeMumbrum and Jackson (1956b) attributed their results to only part of the positive charge on copper being satisfied by the negatively charged exchange complex. Bingham et al (1964), who worked with higher copper concentrations, explained excess retention as precipitation of $Cu(OH)_2$ in the clay system, while Kabata-Pendias (1968) suggested that the formation of complex ions ($CuOH^+$, $CuCl^+$) predominated over precipitation.

Bingham et al (1964) and other workers since (Kisk and Hassan, 1973; McBride and Mortland, 1974), have found no evidence of specific sorption of copper although El Sayed et al (1970) stated that standard enthalpy changes indicate that copper ions are bonded more strongly than those of calcium to clay surfaces. Kisk and Hassan (1973) reported that kaolinite and montmorillonite released large amounts of adsorbed copper, but vermiculite retained it more strongly. According to Kabata-Pendias (1968), however, sorption by kaolinite was low relative to that of other clays and the copper was least exchangeable.

The majority of work with clays is intended to elucidate adsorption mechanisms and often involves saturation with copper, as the evidence of precipitation indicates. Epstein and Stout (1951) attempted "to approach conditions of more direct physiological significance in soil plant interrelations" by growing plants in a suspension of bentonite only partially saturated with copper. Below copper levels of about 0.1 - 0.2% of the clay exchange capacity, uptake by plants was a linear function of the degree of saturation of the clay with copper. Above this level, the rate of change in uptake decreased. Unfortunately, solution concentrations of copper

were not determined.

McLaren and Crawford (1973b) concluded that specific adsorption of copper by clays, although it occurred, would not be a major part of total specific adsorption in most soils.

McBride (1976), like DeMumbrum and Jackson (1956a), found that montmorillonite effectively removed copper from solutions of low concentration. The adsorption was not, however, in preference to other divalent cations but McBride suggested that in some soils it could rival adsorption by organic matter. He also found bonding sites to be non-homogenous. It is thus undesirable to extrapolate from the work previously described to the availability of copper in soils *in situ*, where the amounts and concentrations of copper are much smaller.

2.4.5. The association of copper with soil oxides

The importance of oxides of iron and manganese as adsorbers of copper has only relatively recently been realised, partly because they form only a small percentage of soil mineral matter. As well as in discrete particles, they occur in soils as occlusions and coatings of other particles (McKenzie, 1967), with the result that their adsorption properties may previously have been wrongly attributed. In spite of their low abundance, a mean of 15% of soil copper is associated with oxides (from data of McLaren and Crawford, 1973a). This is achieved by copper levels such as 44 - 210 $\mu g\ g^{-1}$ in manganese nodules, which contain less active soil materials as well as oxides (McKenzie, 1967), and 300 - 600 $\mu g\ g^{-1}$ in iron oxides of podzolic soils containing 10 - 15 $\mu g\ g^{-1}$ total copper (Grimme, 1968).

McKenzie (1967) found that manganese minerals, in the presence

of an excess of calcium ions, are capable of almost complete removal of copper from dilute solutions and Grimme (1968) obtained high adsorption of copper by synthetic goethite and other forms of iron oxide in KNO_3 solution. Also working with goethite, Forbes et al (1976) found that in 25 minutes, 20 µg of material sorbed 90% of the copper supplied (82µg) at pH 5.9 but only 9% at pH 4.7

Grimme (1968) concluded that adsorption by goethite occurs probably by exchange with H^+ on the oxide surface, while McKenzie (1970) divided adsorption by manganese oxides into two phases. Rapid adsorption occurs, again by displacement of H^+ at the surface. At the same time, copper can slowly diffuse into the surface layers of the crystal lattice and replace Mn^{2+}, since the former has a greater crystal stabilisation energy.

Forbes et al (1976) found that, of the metals studied, copper had the lowest affinity for the surface of goethite. This is compatible with the suggestion (McLaren and Crawford, 1973a) that manganese dominates adsorption, contributing up to 30% of the soil's ability to adsorb copper, but the more abundant iron is responsible for occlusion. This is the growth of the particle or coating by deposition of further oxide material, which will isolate adsorbed metals. Occluded copper might, however, become available again under reducing conditions (McLaren and Crawford, 1973a) or by the action of organisms (Bromfield, 1958).

Desorption studies show that sorption by manganese oxide is only partly reversible and the amount extractable with 2.5% acetic acid was less after 7 months (McKenzie, 1967). It has been suggested (Jenne, 1968) that the control of ionic levels in natural waters is

by oxides but this is unlikely to be the only mechanism.

2.4.6. Interaction of soil components in copper adsorption

Since the different colloidal components are present together in the soil, interaction is not unlikely. Bodenheimer and Heller (1967) report that the presence of copper enhanced sorption of amino acids by montmorillonite, leading to stable clay-copper-amino acid associations. Also Jenne (1975) states that the role of clay-size minerals is not as a sink for trace elements but as a mechanical substrate for the precipitation of organic matter and secondary minerals. Jenne (1975) also links organic matter and oxides, stating that amorphous oxides incorporate some organic matter and Schnitzer and Skinner (1965) state that organic matter complexes with hydrous oxides. According to Glinski and Turski (1965), the more stable copper-humic acid complexes incorporate oxides.

McLaren and Crawford (1973a) found that the amount of copper specifically bound to organic matter increased with the ratio of organic matter to free manganese. This appears to be competition between materials for copper rather than a true interaction.

2.5. The availability of soil copper to plants

2.5.1. The effect of total soil copper on plant uptake

Total soil copper is a finite quantity and thus straight-forward to measure but its determination in soil samples requires the use of harsh extractants such as HF (McLaren and Crawford, 1973a) or the combination of H_2SO_4, HNO_3 and $HClO_4$ used by Balraadjsing (1972). This treatment is necessary because a considerable fraction of soil copper is incorporated into primary and secondary soil minerals. An estimate of the latter is the copper remaining after extraction of soil with a mixture of ammonium oxalate and oxalic acid, which averages at about 54% of the total (McLaren and Crawford, 1973a; Shuman, 1979). This is not, however, a constant proportion, the former authors having obtained values between 23.7% and 72.2% for different soils.

If a major and highly variable part of soil copper is in such a form, it is obvious that copper can be present in quantity without sufficient being available to plants. Thus total soil copper is a poor measure of availability to plants. However, the converse also applies. The amount of copper in the available pool cannot exceed the total soil content. Thus Purves and Ragg (1962) were able to state that soils with less than 2 ppm total copper are likely, now or soon, to be associated with copper deficiency. More precise assessment can be made from total copper figures only where geological variation is limited to the extent that the size of the residual copper fraction is almost constant. Such a situation would explain the statement of McKenzie (1966), that predictions of copper status based on total soil copper would be accurate in 90% of soils. Where, however, the soil minerals are more varied, a different approach to

assessing soil copper status is required.

2.5.2. Available soil copper

Allison et al (1927) were aware that copper deficiency could result from the element being present in the soil but in a form unavailable to plants and a threefold increase in plant yield as a result of autoclaving indicated to Piper, (1942) that the soil under study was deficient in available rather than total copper. Up to the middle of the century, soil copper appears to have been thought of as being in two distinct forms, available and unavailable. Copper added to soil was divided into these two catagories by Antipov-Karataev (1947).

2.5.2.1. Biological assessment of available soil copper

The earliest assessment of the degree of a copper deficiency was by the comparison of characters of the affected plants, such as their height and grain yield, with those of plants supplemented with copper. When, more recently, less severe cases of deficiency were recognised, copper concentrations in plant material were determined. This approach was encouraged by the absence of any definition of the copper involved other than "plant available copper". Foliage was analysed because of the difficulty of obtaining an uncontaminated root sample. Analysis of the latter would give a better idea of actual plant uptake but it is in the foliage that deficiency symptoms appear and the copper level here does not include inactivated accumulations. Environmental factors, however, influence herbage copper levels to the extent that values obtained are only relative, even for the same variety of plant. Lundblad et al (1949) concluded

that plant analysis could not indicate if a soil required supplementation with copper and it is only in a few specific cases, such as orange leaves (Embleton et al, 1978) that rigid interpretation of values has been found to be possible.

In order to obtain, for other species, herbage values that are of greater use, it is necessary to reduce environmental variation. This is usually done by growing plants in pots in a greenhouse. This has a major disadvantage as a technique for assessing available soil copper in the time taken, which is about two months. If, instead of mature plants, seedlings are analysed, the time required is considerably reduced and the effects of environment are correspondingly decreased further. The material analysed is of a less complex organism in which shoot copper content will better reflect root uptake although, of course, the values obtained will not be the same as those from more mature plants. Known as the Neubauer technique, it consisted for Beyers and Hammond (1971) of growing barley seeds in a mixture of purified quartz sand and the soil to be assessed. Aerial growth was analysed after seventeen days. Disadvantages of this technique are that the high plant density results in a large demand for soil copper and thus solubilisation of copper beyond normal levels (Petruzzelli and Guidi, 1976) and, because of the short growth period, the copper content of the seed could have a significant effect of the herbage content measured.

In 1939, a technique was developed which enabled the assessment of copper availability using a small sample of soil in the laboratory, although it also was a biological method. The colour, varying between yellow and black, of the spores of the mould Aspergillus niger

depends on the supply of copper. A mixture of 1g air-dried soil and 40 ml purified medium was inoculated and, after four days at 30°C, the spores were compared with those grown in standard solutions. With this method absolute values were possible. Soils containing less than 0.6 μg Cu g^{-1} by this estimation were associated with pronounced symptoms of deficiency while 2 μg g^{-1} or higher gave normal plant growth (Mulder, 1950; Gerretsen, 1952).

The Aspergillus technique unfortunately estimates the availability of copper to an organism differing in nutrition and metabolism from higher plants, which is able to utilize completely copper supplied in various inorganic and organic forms (Gerretsen, 1952), although not that fixed by peat (Mulder, 1950). Assessment of "available" soil copper using Aspergillus took only four days but the development of chemical extraction methods eventually replaced this bioassay technique.

2.5.2.2. Chemical assessment of available soil copper

The need was stated for "a simple and quick method of determining the available copper in the soil" (Gilbert, 1948) or, more realistically, "a partial extraction method extracting only the more readily soluble fraction of the copper in the soil" (Lundblad et al, 1949). The latter authors were among the first to attempt to determine available copper by using a chemical extractant, using the nitric-perchloric digestion technique normally applied to plant material. Others since have tried most reagents used in soil analysis (table 2.3.)

All chemical methods involve shaking soil with an extractant solution and determination of copper in the separated extract. Each combination of reagent, soil to solution ratio and shaking time,

Table 2.3.

Chemical extractants which have been recorded, in at least 3 papers, as being used to estimate available soil copper, plus references

Acetic acid, generally 2.5%	:	Beyers and Hammond, 1971 (McLaren and Crawford, 1973a- fractionation)
Acetic acid + ammonium acetate, both 0.5N, pH 4.7	:	Merodio, 1970
Acetic acid + sodium acetate, pH 4.8 (Morgan's reagent)	:	Vijay et al, 1973
Ammonium acetate, normal, pH 4.8 & pH 7	:	Gabriels and Cottenie, 1976 Dwivedi and Shanker, 1977
Ammonium oxalate, 0.2M, pH 3	:	Duque Macias, 1973
Calcium chloride, 0.1-1.0 N	:	Sabet et al, 1975
Citric acid, 1% and 2%	:	Dwivedi and Shanker, 1976, 1977
D.T.P.A. (Diethylenetriamine-pentaacetic acid) e.g. 0.005M	:	Brown and Boer, 1976
E.D.T.A. (Ethylenediaminetetra-acetic acid), 0.01 - 0.05M, pH 4.5 and 7.0	:	Dwivedi and Shanker, 1977
Hydrochloric acid, 0.1N	:	Dwivedi and Shanker, 1977
1.0N	:	Zietecta, 1977
Magnesium chloride, 1N, 2N	:	Minami et al, 1972; (Shuman 1979 - fractionation)
Nitric acid e.g. 0.5N	:	Zietecta, 1977; Krähmer and Bergman, 1978
Sodium or potassium pyrophos-phate, 0.1 - 0.2M	:	Grewal et al, 1969
Sodium hydroxide e.g. 0.1N	:	Gupta and Mackay, 1966
Water	:	Dwivedi and Shanker, 1976

however, removes from one soil a different amount of copper. It is necessary, therefore, to compare the results obtained using different extractants with values for plant uptake from the same soils, in order to justify any one extractant. Even when this is done, the choice of the most appropriate extractant is still based on correlation, because the majority of extractants remove considerably more copper from the soil than is taken up by a crop (Øien, 1966). The latter is partly due to the high liquid to soil ratio necessary for extraction, which gives the extractant far greater contact with the soil than a plant has. The principal reason, however, is that when chemical extraction techniques were first developed, an important requirement of any extractant was that it removed sufficient copper from the soil to allow analysis by the techniques available. For this reason, acids were preferred to ammonium acetate and water was dismissed as an extractant because of the small amounts of copper removed (Ranadive et al, 1964).

In the seventies, however, the same extractants were still being evaluated (Arora et al, 1971; Duque Macias, 1973; Fachinetti et al, 1973; Zietecta, 1975; Dwivedi and Shanker, 1976) although, some years before, copper levels in water extracts had been sucessfully determined by Gupta and Mackay (1965) and soil solution copper contents measured by Geering and Hodgson (1966). In spite of this, the extractants first used have persisted. There have been only slight modifications to the range, such as the introduction of DTPA (Diethylenetriaminepenta acetic acid) (Dolar and Keeney, 1971). The small size and constancy of the range of extractants means that, in spite of extractant comparisons to find the one most suitable for

local conditions, some comparison of values from different sources is still possible.

The most widely used chemical extractant is EDTA. It does, however, have some disadvantages. Chelating agents, such as EDTA, are most efficient at removing the copper associated with organic matter (Zietecta, 1975) and it has been suggested that large amounts of clay inhibit the extraction (Martin and Reeve, 1957). Two examples of EDTA giving a poor assessment of copper availability are quoted by McKechnie : In field tests in W. Africa, EDTA accounted for only 38% of the variability in crop copper uptake and, in Germany, plants were able to utilize copper in slag which was not extractable with EDTA. In the majority of cases, however, correlation between extracted soil copper and plant uptake is reasonable. In addition, there is a large body of published data obtained using this extractant which, in spite of some variation in extraction conditions used by different workers, allows comparison of results.

2.6. Factors affecting the availability of copper

The use of chemical extraction techniques to determine the amount of available soil copper implies that the amount of copper involved is a fixed characteristic of the soil. That this is not so is demonstrated by the variation in the values obtained according to the extractant used. The quantity of "available" copper is also modified by other factors. While some variation occurs naturally, the largest changes usually result directly or indirectly from the activities of man. Often only the effect is certain because of the difficulty of separating possible causative factors.

2.6.1. Drainage and climate

Comparison of poorly drained areas with nearby well-drained land showed that amounts of EDTA-extractable soil copper and copper levels in red clover were higher in the former. The differences were attributed to ferromagnesian minerals giving different products of weathering according to environmental conditions (Mitchell et al, 1957; Swaine and Mitchell, 1960). Caldwell (1971b) observed fields on peat where copper deficiency was severe generally but absent from poorly drained patches and mentioned a case of copper deficiency occurring on peat when the water table was lowered. The latter case requires a different explanation to that given above, as does the decrease in exchangeable soil copper and manganese obtained by Cheng and Pesant (1977) as a result of aeration in a pot experiment. The quick responses suggest a direct influence of moisture content on copper availability.

If there is a true equilibrium between solid and solution phase copper, the copper concentration in solution will be independent of the soil moisture content. A given volume of soil will thus contain a larger amount of copper in solution than a dry soil and thus a larger amount of copper immediately available to plants. This may partly explain the observations above.

Soil water status and its effects may be one of the factors causing variation in the extent of copper deficiency in different years and seasonal variation in available soil copper. Caldwell (1971b) found that copper deficiency was often severe in dry, sunny years but absent in moist seasons and Priemskaya (1969) determined that, in fen peat, the amount of "mobile" copper was highest in May.

2.6.2. pH

Although this is a soil characteristic it is frequently modified by man.

Svanberg et al (1949) showed that fixation of copper by soil increases rapidly between pH 2 and pH 4.5, with no maximum below pH 6-7. McLaren and Crawford (1973b) and Cavallaro and McBride (1978) also found that the adsorption of copper by soils increased with pH. The latter suggested that low pH values increase the number of functional organic groups associated with protons or Al^{3+}. A similar trend has been found in studies of adsorption by individual soil materials including organic matter (Broadbent and Ott, 1957; Northmore, 1959), clays (Bingham et al, 1964; Kisk and Hassan, 1973) and oxides (McKenzie, 1967; Grimme, 1968; Forbes et al, 1976).

In spite of its effect on adsorption, variation in pH causes

only small changes in the availability of copper to plants (Lundblad et al, 1949) although the trend is similar, as Piper (1942) found by lowering the pH of soils used in a pot experiment. Truog (1946) indicated diagrammatically that availability of copper and zinc is at a maximum between pH 5 and pH 7. A similar diagram (Lucas and Davis, 1961) indicates maximum availability of copper, in organic soils, between pH 5 and pH 6.5, with a rapid decrease below pH 4.5. Van Luit and Henkens (1967), however, found plant uptake of copper to be largest at soil pH values between 4.2 and 5.7. Variation in the availability of copper with pH may be limited by much of the copper in solution being complexed by soluble organic molecules (Loneragan 1975) in a manner which restricts its uptake by plants. This would also explain the low availability of copper in soils of high pH, since there is a high degree of complexing under these conditions (McBride and Blasiak, 1979).

Mitchell et al (1956) obtained a slight reduction in plant uptake of copper after liming soils but when copper also had been added the change was negligible. Liming has also been found to increase plant copper uptake indirectly, by improving the rooting of a crop (Caldwell, 1971b).

2.6.3. Plants and other organisms

The plant is often not considered as a factor affecting copper availability but as simply a consumer, removing copper from the soil solution. There is evidence, however, for inclusion of the plant as an external factor acting on soil. Firstly, copper transported in roots from the deeper parts of the soil enriches the surface horizons

on the decay of the plant residues. An increase in availability of copper after a grass crop has been attributed to this cause (Steenbjerg, 1950).

Nielsen (1976b) obtained, over a 100 day period, samples of soil solution from pots in which barley was growing. Whereas without plant cover copper levels remained constant, they were considerably increased by plant growth. Optical absorbance of the solution, which was taken as an estimate of dissolved organic matter, increased similarly. At least part of this organic material comes from the plant as root exudates. In tea plants these include appreciable quantities of the chelating agent malic acid (Jayman et al, 1975) and root washing of oat plants contained substances which dissolved a biologically-formed manganese oxide, making manganese available to the plants (Bromfield, 1958). Such oxides contain large amounts of copper (McKenzie, 1967) which would also be chelated and present in the soil solution. If the substances complexed by root exudates are utilized by the plant, it has increased its own nutrient supply. Solution of apparently insoluble material in this manner would explain the field trials in which copper slag gave results comparable to or better than the equivalent amount of $CuSO_4$ (McKechnie).

It is also possible that organisms other than the plants themselves are responsible for increasing availability of soil copper. The mould Aspergillus niger is capable of completely solubilising copper supplied in various forms (Gerretsen, 1952) and soil bacteria producing 2-ketogluconic acid released calcium from compounds of low solubility, including a silicate (Duff and Webley, 1959). In the case of a mycorrhizal infection, copper is supplied directly to the

plant (Hewitt and Smith, 1975) but beneficial effects of some free soil organisms may be cancelled out by the activity of other micro-organisms. Phosphate release from minerals has only been demonstrated *in vitro* (Barber, 1969) and, in the absence of a germicide, the ability of root washings to dissolve MnO was rapidly lost (Bromfield, 1958). The occurrence of copper deficiency in barley grown on a chalk soil after the ploughing in of kale (Davies *et al*, 1971) may well be due to the competition for available copper provided by the micro-organisms breaking down the kale, especially since this plant has a low copper content (Alderman, 1968). There is also the possibility of chelates, exuded by micro-organisms, competing with roots for nutrient ions (Brown *et al*, 1960).

2.6.4. Additions of copper to the soil

Applications of copper to deficient soils combat deficiency by increasing available levels (Dunat, 1975). Some of the added copper may become part of less-available pools but concentrations in plants are enhanced for at least 18 years (Reith, 1975). The response to added copper varies with species. Levels in clover are considerably elevated, while the response of ryegrass is limited (Mitchell *et al*, 1957).

In France, Bordeaux mixture has been applied to vines for over a century. The effect of these copper additions has been cumulative, giving soil copper contents such as the value of 320 $\mu g\ g^{-1}$ quoted by Boischot and Quillon (1952). Citrus orchards in the U.S.A. have also received regular copper applications and an induced iron deficiency has been reported on coarse-textured soils (Reuther and

Smith, 1953).

Macroelement fertilizers contain between 1 and 100 μg Cu g^{-1} (Mitchell, 1964). However, a fertilizer containing 10 μg Cu g^{-1}, applied at a rate of 100 kg ha^{-1}, will only supply 1 g Cu ha^{-1}, equivalent to an increase of 0.004 μg g^{-1} in soil copper content (assuming 2.5 kg ha^{-1} ≡ 1 μg g^{-1}). Thus fertilizers do not provide a significant copper supply.

The mining of copper and industries processing and utilizing the metal have caused considerable contamination of the areas in which they take place. Purves (1968) obtained a mean of 56.3 μg Cu g^{-1} from 45 urban soils compared with 15.5 μg g^{-1} for rural soils. The amount carried in the atmosphere is, however, small. Archer (1968) determined that 67 g ha^{-1} $annum^{-1}$ fell close to a power station. At this rate, with no losses, it would take 1,500 years to achieve the level of pollution quoted above.

Sewage sludge and slurry, applied to agricultural land, provide a fertilizer input. The former may contain copper but also less desirable heavy metals. As a result of diet supplements of copper, pig slurry contains in the order of 675 μg Cu g^{-1} dry matter (Batey et al, 1972) but only a fraction of this may be available to plants (Kneale and Smith, 1977). The long-term effect is unknown.

2.6.5. Removal of copper from the soil

The crops and animals produced on farms contain copper, which is consequently lost from the soil. The loss is less than the total content of the organisms, because animals contain copper imported in concentrates and plant residues, including the root system, usually

remain in the field. Root crops, although physiologically different from true roots, still remove from the soil a similar amount of copper to a barley crop (Fried and Broeshart, 1967). The amounts of copper involved are small relative to total soil copper levels but they form a continual and increasing loss of available copper from the system.

2.6.6. The application of fertilizers

Variations in levels of elements other than copper are not necessarily the result of adding materials to the soil but the majority of observations of interaction have been made after the addition of nutrients. Effects observed in plants may result from interactions in either plant or soil.

2.6.6.1. Nitrogen

High rates of nitrogen often increase the copper content of grasses (Alderman, 1968), yet the application of inorganic N can aggravate melanism in wheat (Davies et al, 1971). The copper content of clover is not, however, affected, (Hemingway, 1962) although the application of nitrogen tends to reduce the clover content of a sward, possibly because of increased competition from grasses. Thus the overall herbage copper concentration is actually lowered by moderate nitrogen additions (Alderman, 1968).

It has been suggested that an association between Cu and N during transport in the phloem is responsible for some of the observed interaction (Hill et al, 1978).

2.6.6.2. Phosphorus

The literature concerning the effect of phosphate on copper

availability is contradictory. Mortvedt and Osborn (1977) reported that the application of 2,000 $\mu g\ P\ g^{-1}$ caused a temporary increase in soil solution copper levels and Sapek and Sapek (1976) found a positive correlation between copper and phosphate levels in grasses, yet Tarasov and Kovalenko (1972) found that high rates of phosphate decreased the "mobile" copper content of the soil and the copper content of apple leaves. Similarly, phosphate decreased root copper levels in citrus (Plessis and Burger, 1972) but it had no effect on the copper content of grass or clover (Hemingway, 1962).

2.6.6.3. Potassium

According to Hemingway (1962), additions of potassium to soil had no effect on the copper content of grasses and clover but Sapek and Sapek (1976) found that, in 11 out of 14 plant species, there was a significant negative correlation between plant copper and potassium contents.

2.6.6.4. Manganese, Zinc and Magnesium

Copper utilization by plants is favoured by adequate manganese, which in some plants is assimilated only if zinc levels are sufficient (Buckman and Brady, 1960). Sapek and Sapek (1976) found relationships between plant contents of both zinc and magnesium and plant copper content.

2.7. The importance of the soil solution in plant copper supply

The availability of soil copper to plants has been considered above in terms of the total amount of copper taken up by the plant or the amount extracted from soil by a particular chamical reagent. In addition, the rate at which the plant takes up copper from the soil is also an important aspect of availability.

Although the actual uptake mechanism is unclear, plant roots are surrounded by the soil solution and uptake of copper will be from this medium. Regulation by the plant of this process is unlikely, except possibly as a protective measure against considerably elevated solution concentrations. Thus the rate of uptake of copper by the plant at any moment in time will normally depend on the copper concentration in the soil solution.

The copper concentrations involved are far lower than those in chemical extracts of soils but in recent years suitable techniques have been developed for their accurate determination. The problem is to obtain a sample of the soil solution without disturbing equilibria between it and the solid phase of the soil, which any addition of liquid would do.

Suction methods, when used in the field or laboratory, suffer from the disadvantages of imperfect contact with the soil and the possibility of adsorption of copper by the apparatus (Richards, 1941). In a greenhouse experiment MacLeod (1964) alleviated the former problem by installing a fritted filter, capable of being attached to the suction apparatus, in each pot as it was filled with soil. Nielsen (1972), who used a similar technique, prevented the liquid

junction, between soil and filter, from breaking at low moisture levels by coating the filter with silica gel.

Ideally, laboratory extractions are carried out using fresh soil samples. Adding water to soil immediately before removing "soil solution", as Bradford et al (1971) did, is especially likely to result in the system sampled not being at equilibrium.

The technique of displacing soil solution from moist soil packed in a column by using liquid (Hodgson et al, 1965, 1966) or gas (Mortvedt and Osborn, 1977) was developed by Burd and Martin (1923), who also demonstrated the homogeneity of the displaced solution. The success of the method depends, however, on the soil being at a suitable moisture content and skilfully packed into the column (Richards, 1941; Adams, 1974). Also, in some soils, displacement can be extremely slow, allowing changes in the solution as it passes through the soil.

Centrifuging has only been widely used since the designing of a suitable apparatus by Davies and Davies (1963). Solution samples can be obtained in less than 30 minutes and no preparation of the soil is necessary. Several of the values listed (table 2.4.) were determined using soil solution obtained by centrifuging.

It is possible to extract from the soil, using the techniques named, water that is not available to plants. Burd and Martin (1923) suggested that only the outer layers of the soil moisture content are involved in displacement and Hodgson et al (1965) removed 40% of the soil moisture content in this way. The use of pressure, however, increases the yield (Burd and Martin, 1923) and high pressures could remove, from micropores, water of a different ionic composition to

Table 2.4.

Determinations of copper in soil solution

Author(s), date	Extraction method	μg ml^{-1} in soln	Notes
Boischot and Quillon, 1952	unspecified	0.244	Vineyard regularly treated with Bordeaux mixture - total soil copper 320 μg g^{-1}
Hodgson et al, 1965	displacement with $CaCl_2$ or KBr soln	0.0031-0.0180	10 air-dry mineral soils incubated 2-3 weeks at ± 0.1 atmosphere water tension
Hodgson et al, 1966	displacement with $CaBr_2$ soln	0.0035-0.0392	20 air-dry calcareous soils, incubated 2 weeks at ± 0.15 atmospheres water tension
Mercer and Richmond, 1969	centrifuged at 1800g for 15 min.	0.0240-0.0570	11 air-dry soils, maintained at field capacity for 7 days
Zmijewska and Minczewski, 1969	centrifuged for 15 minutes	0.0780	black earth; air-dry soil, moistened until a glistening surface of the soil was obtained 1 hour before centrifuging
		0.0110	brown earth
Bradford et al, 1971	buchner funnel	0.01-0.2	saturation paste of one soil, extracted 68 times: mean 0.04 μg Cu g^{-1}
Kabata-Pendias, 1972	centrifuged for 20 mins. at 3,000 rpm	0.135	rendzina; moistened soils incubated at 25°C for 3 days
		0.078	granite soil
		0.028	basalt soil
Nielsen, 1972	suction filters buried in plant pots	0.003-0.050	
Yamasaki et al, 1975	centrifuged at 14,000 rpm for 30 minutes.	0.0368	alluvial soil; "Sampled under field moisture conditions"
		0.0228	pumice soil
		0.0527	volcanic ash
Mortvedt and Osborn, 1977	expressed with N_2 gas at 7 kg cm^{-2} for 30 mins.	0.01-0.22	

the "available" water in macropores (Gillman, 1976). It is also possible to exceed the "available" moisture range of 0.1 - 15 bar with many centrifuges. This was in fact done by Yamasaki et al (1975) and could explain the relatively high values obtained (table 2.4.).

Allowing for the fact that some of the solutions sampled would not have been at equilibrium with the soil, the range of copper concentrations in natural soil solution appears to be from about 0.003 $\mu g\ ml^{-1}$ to 0.06 $\mu g\ ml^{-1}$. Other findings confirm this level. Solution culture provides a similar plant environment to that formed by the buffered soil solution. In this artificial situation, the copper concentration is critical if toxicity is to be avoided. The optimum range for plant growth is between 0.02 and 0.05 μg Cu ml^{-1}, although, in the presence of even the low adsorptive capacity of sand, this needs to be increased tenfold (Hewitt, 1952). Also Beckwith (1958) calculated that, if all the copper in oat plants is transported to the roots by the soil solution, then the concentration in that solution, calculated from the ratio of metal uptake to the volume of water transpired, is in the order of 0.01 μg Cu ml^{-1}.

The concentration of copper in solution is low but the requirements of plants are also small. Hodgson et al (1966) calculated that a plant content of 3 μg Cu g^{-1} could be supplied entirely by a transpiration stream containing 0.006 μg Cu ml^{-1}, if none of this copper was excluded from the root and the transpiration ratio was 500 g water g^{-1} tissue. However, it must be remembered that only a small volume of soil is available per plant as a source of its copper-containing solution. The soil solution in this finite volume, in order to meet transpiration needs over the whole growth period of

the plant, will have to be replenished. The water is usually replaced by rainfall and the nutrients in the solution, including copper, will be derived from the solid phase of the soil.

A second calculation is possible from figures for nutrient removal from soil by crops (Fried and Broeshart, 1967). In straw and grain, oats remove 98 g Cu ha^{-1} and wheat 35 g Cu ha^{-1}. If one hectare of top-soil weighs 2,500 tonnes and contains 625 tonnes of water, then, to supply a crop, the soil solution needs to contain 0,157 µg Cu ml^{-1} (oats) or 0.056 µg Cu ml^{-1} (wheat). If it actually contains only 0.01 µg ml^{-1} the copper in it has to be replenished during the growth of the crop, 16 times for oats and 6 times for wheat, from copper adsorbed by the solid phase of the soil.

Thus, although the copper concentration in solution indicates the amount of copper which is available at any moment in time, it is by no means a complete assessment of soil copper availability over any period of time. The rate of supply of copper to the soil solution and the capacity of the soil for that supply must also be considered, i.e. the relationship between solid phase copper and that in solution.

It has been shown that copper concentrations in solutions equilibrated with soils (with the possible exception of calcareous soils) are well below the levels that would be expected if they were controlled by the solubility of inorganic copper compounds such as oxides, hydroxides, carbonates or phosphates (Lindsay, 1972; Fassbender and Seekamp, 1976; Cavallaro and McBride, 1978).

Knowledge of a mechanism capable of control and maintenance of the copper concentration in soil solution dates from the conclusion of Wood (1945) that "soil copper exists in an equilibrium between

available, slowly available and extremely slowly available forms". Viets (1962) provided more details of the forms and their relationships. He listed five pools in which micronutrients are found in soils :

(a) water soluble:ionic or adsorbed on suspended colloids

(b) exchangeable cations

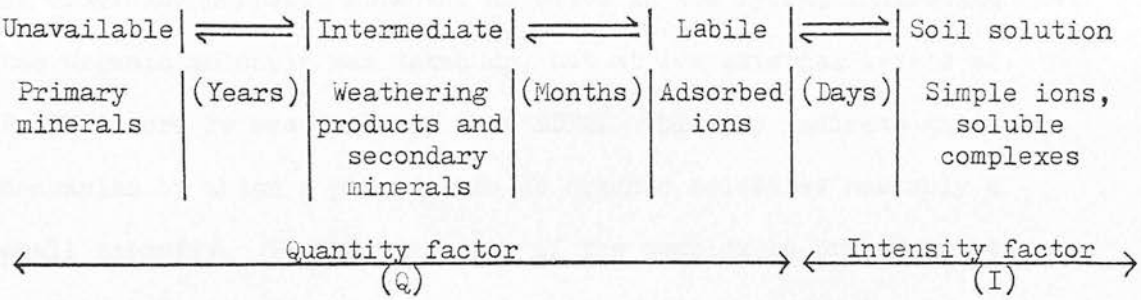
(c) complexed and chemisorbed ions - not desorbed by Ca^{++}

(d) secondary minerals

(e) primary minerals

Pools a,b and c are in reversible equilibrium with one another, with equilibrium being quickly established. These pools contain cations available to plants. Pools a and b are small for copper and thus c is of greatest significance to plants. Pool d equilibrates slowly with the others and e not at all, but it slowly releases cations.

Copper is also present in soils incorporated in organisms and their residues and occluded in soil oxide material (McLaren and Crawford, 1973a). A simplified summary of the forms and relationships, including the time dimension, has been given by West (1979) :



It is likely that the level of copper in the soil solution is regulated by adsorption and desorption, essentially by the various colloidal soil materials, according to the above scheme (McLaren and Crawford, 1973b; Quirk and Posner, 1975; Fassbender and Seekamp, 1976).

The system described is increased in complexity by the fact that

a large part, up to possibly 99%, of the copper in soil solution is organically complexed (Hodgson et al, 1965; Cavallaro and McBride, 1978). The presence of complexed copper increases the amount of copper in solution, since it is only the solution level of ionic copper which is determined by equilibria with the solid phase. Hodgson et al (1966), suggested that high solution copper levels, resulting from a high degree of complexing, might explain why copper deficiency is rare on calcareous soils of the western U.S.A., in spite of low ionic copper levels. This, however, assumes that both complexed and ionic copper can be utilized by plants. Mercer and Richmond (1969, 1970, 1971) found that, in soils known to give rise to copper deficiency, a large proportion of the solution copper was complexed by organic structures of molecular weights greater than 5,000 and of high stability. In this form copper is probably unavailable to plants.

Experiments have been carried out on the uptake of FeEDTA by maize (Beckett and Anderson, 1973), which may be similar to the uptake of complexed copper. EDTA was detected in the xylem, indicating that the organic molecule was taken up, but at low external levels of FeEDTA, more Fe was taken up than EDTA. This may indicate that the mechanism by which a plant takes up organic molecules has only a small capacity. It also implies, if the complex is broken up at the soil — root interface, an accumulation of complexing molecules at this site, which could compete with the roots for copper (Brown et al, 1960) and thus counteract, to some extent, the increase in mobility of copper resulting from complexing (Nielsen, 1976b). Under certain conditions, however, equal amounts of chelate and the associated

copper can be taken up by a plant and reach the shoots (Beringer, 1963).

2.8. Summary

It is evident from this review of the literature that, in spite of the considerable attention the subject of soil copper has received, much is still unknown about the factors controlling the supply of copper to plants.

3. EXPERIMENTAL WORK

3.1. Introduction

3.1.1. The present approach to the assessment and treatment of copper deficiency

The amount of available copper in a soil is commonly assessed by extracting a sample with EDTA solution. Where the result of analyses indicates a need for copper supplementation, various types of treatment may be possible : -

a) soil treatment

b) treatment of crops by spraying foliage

c) dosing or injection of livestock

d) providing livestock with mineral supplements

Although the amount of copper supplied varies according to the type of treatment, each treatment usually involves the application of copper at a standard rate.

3.1.2. Possible improvements in treatment

All the treatments listed above involve the addition of copper, whether to soil, plant or animal. In theory, it should be possible to increase the availability of native soil copper by chemical treatment but suitable compounds would also be likely to increase the availability of other heavy metals to toxic levels. Another approach, of breeding plant varieties to maximise intake or utilization of soil copper, is unlikely to be used because of the need for high yields. In any case, this approach would not solve the problem of

depletion of copper from the soil as a result of removal in plant material. It is therefore necessary to make additions of copper to the soil.

Supplying additional copper directly to the organism, although often beneficial, is not a satisfactory substitute to providing the organism with an adequate long-term supply of copper. Spraying crops when deficiency symptoms show does not necessarily compensate for the associated check in growth. Also, it is often difficult in practice to supplement the diet of a grazing animal and injecting selected animals with copper is likely to be either ineffective or uneconomic, depending on the proportion of the stock treated. In some cases, however, such direct treatment of animals may be the only possible cure or preventive measure, especially where the deficiency is induced rather than due to a simple lack of copper.

Where copper is added to the soil, it is unlikely that a single rate of copper is the ideal treatment for deficiency in all mineral soils. Although an application at the recommended rate will benefit crops and animals on most deficient soils, the optimum rate, in many cases, could be several times larger. Increasing the standard rate is not, however, advisable, because of the small return likely, on many soils, for the large expenditure involved. It is thus desirable to be able to predict the response of a soil to copper by routine laboratory analysis. For this to be possible, much more needs to be known about the interaction of copper with the various materials of which soils are composed, at the concentrations of copper found in normal soil solutions.

3.1.3. Possible improvements in soil assessment

In addition to its other disadvantages, chemical extraction is not appropriate for assessing availability when the latter is determined by equilibria between soil components. Chemical extractants tend to remove large amounts of copper including much which is of low availability and they fail to simulate the "progressive dynamic equilibrium" (Gerretsen, 1952) which exists as a result of the withdrawal of copper by the plant from the soil solution. Instead, the soil-extractant system proceeds to a static equilibrium.

Although the existing methods for the assessment of available soil copper have serious disadvantages, they are unlikely to be replaced in the near future. Even when routine determination of soil solution copper levels is possible, the values obtained will be of little use except when quoted in combination with supply and capacity characteristics of the solid phase. Chemical extraction techniques have the advantage of representing soil copper availability with a single value. Thus a standardised technique giving reproducible results using, for example, EDTA, has a useful role in characterising soil copper status. However, from this base, there is considerable scope for improving the interpretation of the value obtained, by considering the nature and proportions of the component materials of the soil and the way in which they affect copper availability.

3.2. Routine methods used in research

Experimental work was carried out with the aim of filling, to some extent, the gaps in present knowledge which have been indicated above. The different aspects investigated are described and discussed in subsequent sections of this work.

3.2.1. Contamination prevention

Copper is sufficiently abundant in the laboratory environment for contamination to be a problem in the analysis of low solution concentrations of copper. In order to minimise contamination, all laboratory glassware and polypropylene apparatus was washed thoroughly, then rinsed with 1N "Aristar" HCl and deionised distilled water (glass-distilled water passed through a column of Biorad Chelex 100 resin in the Ca^{2+} form). The purified water was also used to prepare solutions from "Aristar" or "Ultrar" reagents. The exception was the 0.05M $CaCl_2$ solution used in adsorption work, which was made up in distilled water and then purified using the resin column.

The apparatus employed was chosen with a view to reducing contamination. Glass centrifuge tubes were used for extractions into organic solvent, rather than the more difficult to clean separating funnels. One kind of automatic pipette tip was avoided after the discovery that it increased copper levels in some dispensed solutions. Soil extracts were separated from soil by centrifuging rather than by filtering. This avoided possible contamination or retention (Bolt, 1961) of copper by filter papers and reduced the contact of the sample with glassware.

3.2.2. Soil extraction techniques

3.2.2.1. Extraction of copper with EDTA

4g air-dry soil, ground to pass through a 2 mm sieve, was weighed into a 50 ml polypropylene centrifuge tube. To this was added, by pipette, 20 ml of a 0.04M solution of di-sodium ethylenediaminetetra-acetate (EDTA). The tube was placed on a rotary shaker for one hour and allowed to stand for a second hour. After centrifuging, at 7,000 rpm for 10 minutes, copper was determined in the supernatant by atomic absorption spectrophotometry, using flame atomisation.

Suspicions that the amount of copper extracted by EDTA varied with temperature were confirmed by a series of extractions at temperatures between 10°C and 30°C (figure 3.1.). At higher temperatures the soil extract contained more copper and more organic matter (indicated by a higher optical density at 400 nm), showing that the effectiveness of the extractant varied with temperature. Henceforth, all extractions were carried out in an incubator at 20°C. A rotary shaker was installed and the tubes remained in the incubator for the hour between shaking and centrifuging.

3.2.2.2. Fractionation of soil copper

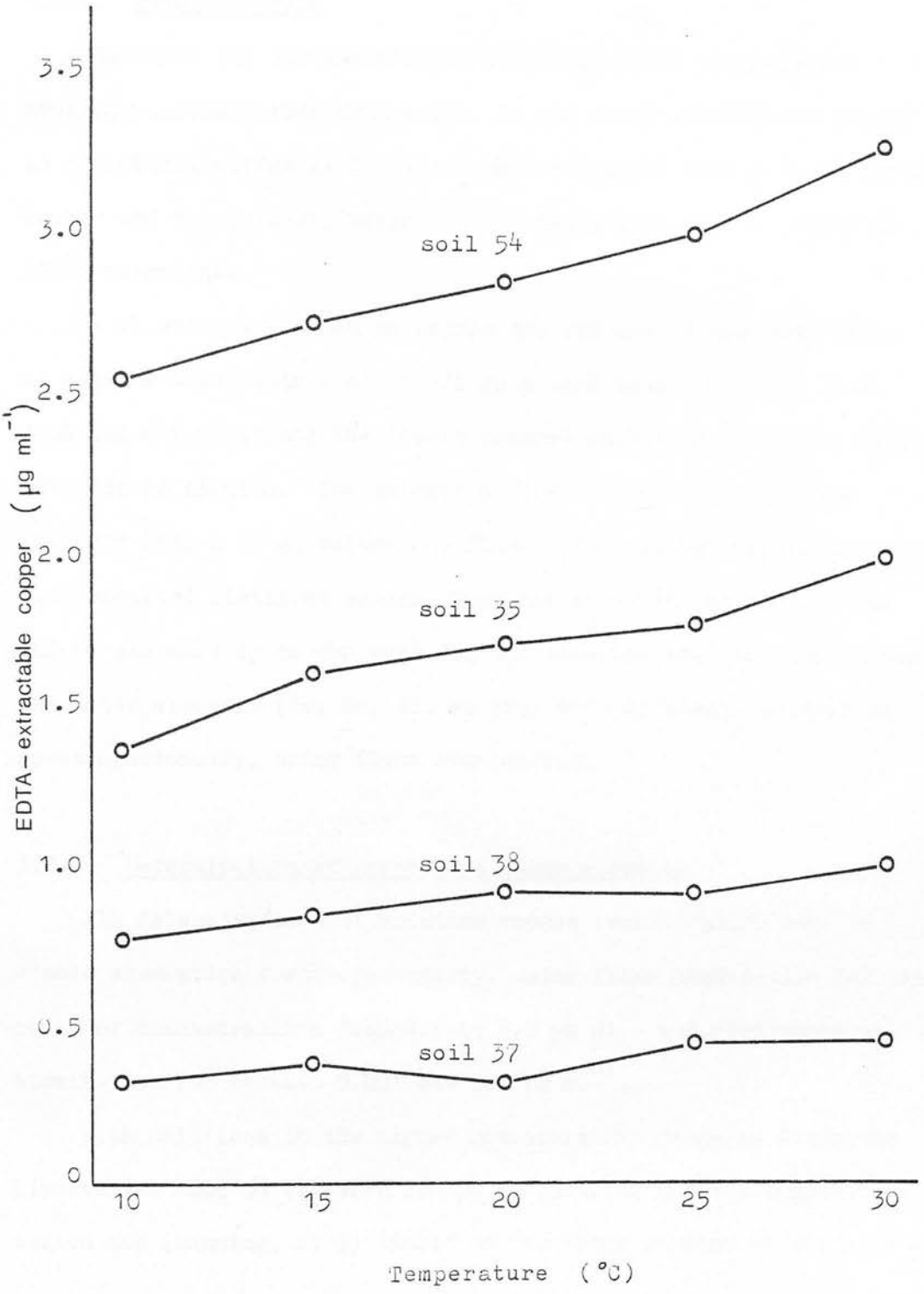
The method used was modified from that of McLaren and Crawford (1973a) (appendix 1)

3.2.2.3. Extraction of free iron and manganese

1g air-dry soil and 50 ml acid oxalate (oxalic acid 0.1M, ammonium oxalate 0.175M), in an evaporating basin, were placed on a steam bath under an ultraviolet lamp for 2.5 hours. The volume was maintained with distilled water. The resulting mixture was filtered into a 200 ml flask. When cool the volume was made up to the mark

Figure 5.1.

The effect of temperature on the amount of copper extracted by EDTA



with water and the iron and manganese contents of the solution were determined using atomic absorption spectrophotometry.

3.2.3. Plant analysis

Material was harvested using stainless steel scissors and avoiding contamination with soil. It was dried at 60°C and ground in a Moulinex coffee mill. About 2g was weighed into a 25 ml pyrex beaker and the oven-dry weight (80°C) determined before ashing at 450°C overnight.

1 ml water was added to settle the ash and it was then taken to dryness twice with 2 ml 6N HCl on a sand bath at 100°C. 5 ml 1.5N HCl was added and the beaker covered and returned to the sand bath for 30 minutes. The extract of the ash thus obtained was filtered into a 20 ml volumetric flask. The residue was washed with hot deionised distilled water. When the solution had cooled, the volume was made up to the mark and the solution analysed for copper and other elements (Fe, Mn, Zn, as required) by atomic absorption spectrophotometry, using flame atomisation.

3.2.4. Determination of copper and other elements

All determinations of solution copper concentration were by atomic absorption spectrophotometry, using flame atomisation for the range of concentrations from 0.1 to 3.2 $\mu g\ ml^{-1}$ and electrothermal atomisation for between 0.001 and 0.1 $\mu g\ ml^{-1}$.

With solutions in the higher concentration range, an automatic pipette was used to transfer 200 µl aliquots of aqueous sample to a teflon cup (Manning, 1975) linked to the spray chamber of the atomic

Table 3.1.

Determination of copper by atomic absorption spectrophotometry

Instrument	:	Varian Techtron type AA5
Wavelength	:	324.8 nm
Slit width	:	0.20 - 0.33 μ
Lamp	:	Pye Unicam Zinc lamp, part 611303
Lamp current	:	5 - 6 mA
Mode	:	normal
Damping	:	A (minimum setting)
Scale expansion	:	Up to maximum, as necessary
Presentation of values	:	Oxford Instruments 3000 series chart recorder, paper speed 2 cm/min ("slow" setting used with carbon rod)
Atomisation	:	(a) air-acetylene flame air at 20 lb/in^2 acetylene at 8 lbf/in^2 gain setting 2-3
		(b) Varian Techtron model 63 carbon rod electrothermal atomiser nitrogen supplied at 10 lbf/in^2 gain setting 3-4

atomiser settings :

phase	voltage setting	time (seconds)
dry	4	25
ash	5.5 - 6.5	15
step atomise	7	2.5

absorption spectrophotometer. Standards were prepared containing between 0.1 and 3.2 $\mu g\ ml^{-1}$ copper in the appropriate extractant solution and aspirated to provide calibration.

When solution copper levels were below about 0.1 $\mu g\ ml^{-1}$ the copper in an aliquot of aqueous solution was complexed with ammonium pyrrolidine dithiocarbamate (APDC) and extracted into the organic solvent methyl-isobutyl ketone (MIBK) (appendix 2). A Varian Techtron model 63 carbon rod electrothermal atomiser was used in conjuction with the atomic absorption spectrophotometer to determine the copper content of 5 µl aliquots of the organic phase. Standards, also extracted into MIBK, provided calibration. Extraction into organic solvent increased sensitivity, allowed a concentration step and enabled eletrothermal atomisation free from the interferences associated with aqueous solutions (Allen, 1961; Yamasaki et al, 1975)

Pyrophosphate extracts of soil, which blocked the burner if analysed in the aqueous form, were also extracted with APDC into MIBK but copper was determined using the teflon cup and flame atomisation.

Calcium, Iron, Manganese and Zinc were determined in aqueous solution by atomic absorption spectrophotometry. A similar technique to that used for copper, using the teflon cup attachment, was employed.

Concentrations of ionic copper (Cu^{2+}), as opposed to total soluble copper, were also determined in solution samples. A cupric ion electrode (Orion 94-29) was used in conjunction with a single-junction reference electrode (Orion 90-01). Electrode potential was measured with a digital millivoltmeter (Orion 701A). Calibration was carried out using standards of known Cu^{2+} concentration. All solutions

were adjusted to the same ionic strength with 5M $NaNO_3$ and stirred while measurements were being made. Values for electrode potential were plotted against the logarithms of the Cu^{2+} concentrations. A straight line calibration was obtained only from 10 down to 0.01 μg Cu^{2+} ml^{-1} but concentrations as low as 0.001 μg Cu^{2+} ml could be determined (figure 3.2.).

3.2.5. The radioactive isotope copper-64

3.2.5.1. Preparation

Radioactive copper was obtained by the irradiation of copper-containing compounds for six hours, at a flux of 3×10^{-12} neutrons s^{-1} cm^{-2}, in the UTR reactor at East Kilbride. Low specific activity copper-64 was obtained by the irradiation of 12 mg solid $CuCl_2.2H_2O$, which was then dissolved in 0.05M calcium chloride. The specific activity on removal from the reactor was approximately 0.4 mCi mg^{-1} Cu.

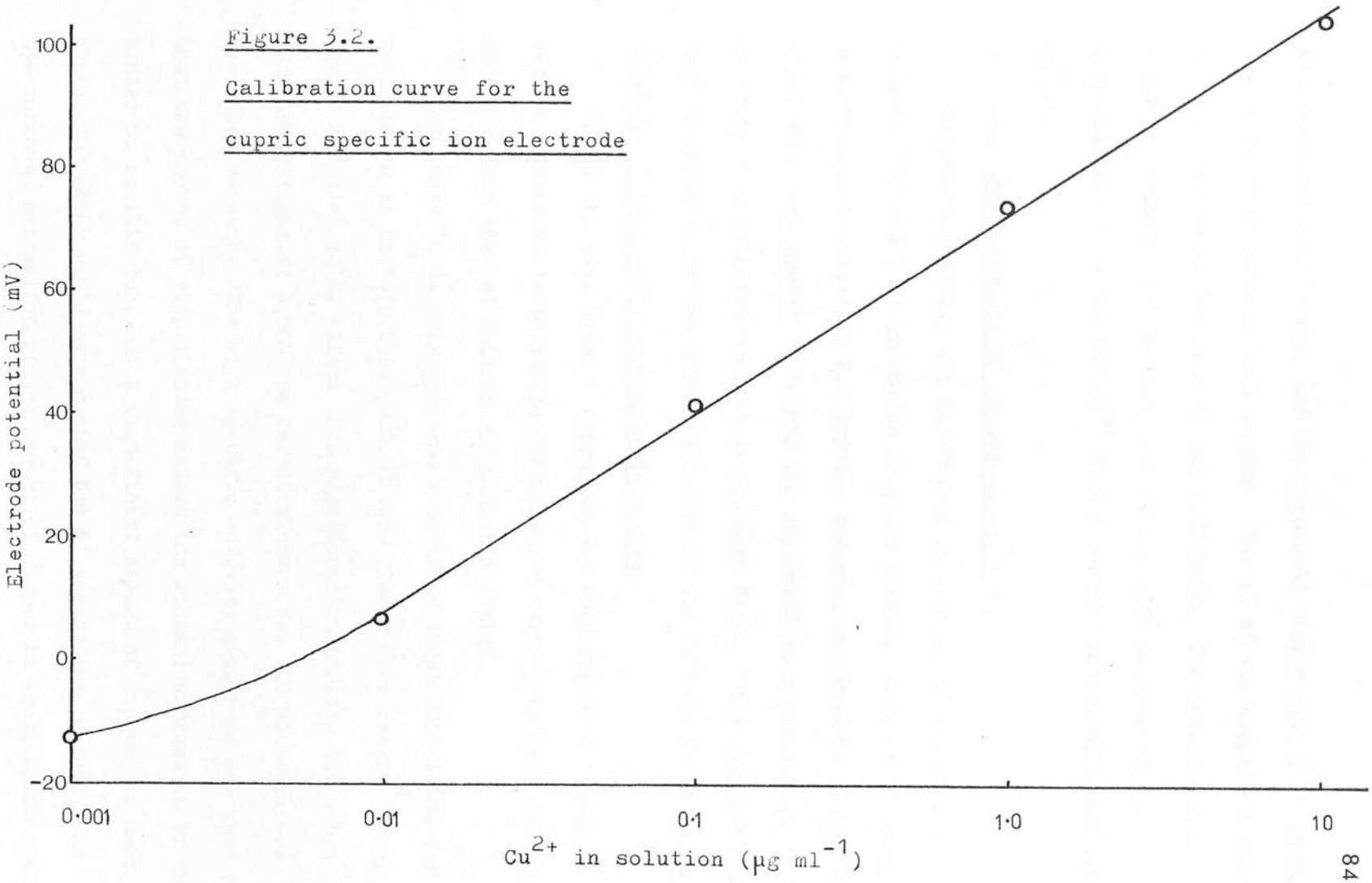
To obtain copper-64 of a higher specific activity, use was made of the Szilard-Chalmers process (Ebihara, 1966). Copper phthalocyanine was first purified by dissolving the solid in H_2SO_4, reprecipitating in deionised distilled water and washing before freeze drying. After the irradiation of 50 mg of the solid, it was dissolved in 0.5 ml concentrated "Analar" H_2SO_4 and reprecipitated by washing into 100 ml water. The copper phthalocyanine was removed using a Millipore GS 0.22 μm filter and the precipitate washed. The filtrate, containing ionic copper-64 was brought to pH 6 - 7 with 2M and 0.2M NaOH.

At this stage the volume was about 300 ml. This was passed through a column containing 0.9 g Chelex 100 chelating resin, 100—200 mesh, in the sodium form. The resin was washed with 100 ml

Figure 3.2.

Calibration curve for the

cupric specific ion electrode



deionised distilled water and the copper-64 eluted with 2 ml 1N HCl and 10 ml water into a small beaker. The pH of the copper-64 solution was adjusted to pH 6.0 with 2M and 0.2M NaOH. The result was a solution concentrated to less than 20 ml, with an activity of approximately 50 - 100 mCi mg^{-1} Cu and copper content of about 0.4 µg ml^{-1}.

3.2.5.2. Determination of copper-64 activity

Copper-64 activity was determined in samples of solution of between 1 ml and 5 ml in volume in glass phials. A scintillation counter with a well-type NaI crystal detector was used to count γ-ray emissions. The number of counts was expressed as a percentage of the activity of a standard counted at the same time. This overcame the need to correct for the short half-life of the isotope (12.8 hours).

3.2.5.3. Isotopically exchangeable copper

One of the ways in which copper-64 was used was as a tracer in determinations of isotopically exchangeable copper, using a method modified from that of McLaren and Crawford (1974).

Duplicate 50 ml polypropylene centrifuge tubes containing 4 g soil and 40 ml $CaCl_2$(0.05M, pH 6.0) were placed on a rotary shaker for 24 hours at 20°C. After this equilibration period, the tubes were centrifuged at 6,000 rpm for 10 minutes and 100 µl copper-64 was added to each. The high specific activity copper-64 was used so that the amount of copper added caused the minimal disruption to the system at equilibrium. If a significant amount of copper had been added, the study would have become one of adsorption, rather than the intended estimation of the amount of copper in equilibrium with the solution phase of the system. In fact, the amount of copper

added was only 0.001 $\mu g\ ml^{-1}$ solution.

After the second equilibration period of 14 hours, the tubes were removed from the shaker and centrifuged again. The proportion of the copper-64 activity remaining in the solution phase was determined and the solution copper concentration obtained by atomic absorption spectrophotometry. The amount of isotopically exchangeable copper in the system was calculated thus :

$$\text{Cu IE (total)} \quad (\mu g\ g^{-1})$$

$$= \frac{\text{Cu-64 (added)}}{\text{Cu-64 (in solution at equilibrium)}} \times \frac{\text{Cu (in solution)}(\mu g)}{\text{weight of sample}(g)}$$

$$= \frac{\text{Solution copper concentration at equilibrium } (\mu g\ ml^{-1})}{\text{Fraction of added copper-64 in solution phase at equilibrium}} \times \frac{\text{Solution volume (ml)}}{\text{Weight of sample}(g)}$$

The amount of isotopically exchangeable copper associated with the solid phase is equal to Cu-IE(total) minus the copper in solution.

3.2.6. Soil materials used in adsorption studies

Details of the individual materials are given in table 3.2. All materials were calcium-saturated by washing with 0.05M $CaCl_2$. Excess calcium was removed by washing with deionised water and the materials were freeze-dried before use.

3.2.7. General soil properties

Values for soil pH were obtained by adding 75 ml 0.01M $CaCl_2$ to 30 g air-dry soil in a polythene beaker. The mixture was stirred and allowed to stand for one hour before stirring again and determining

Table 3.2.

Soil materials used in adsorption studies

Material	Source
Clay minerals (The <2um fraction was used in each case)	
Kaolinite	Supreme - English China Clays
Montmorillonite	No. 27, Belle Fourche, South Dakota } Standard A.P.I. Project 49 clays
Illite	No. 36, Morris, Indiana } Standard A.P.I. Project 49 clays
Oxides	
Synthetic iron oxide	As prepared by McLaren and Crawford, (1973a)
Synthetic manganese oxide	As prepared by McLaren and Crawford, (1973a)
Soil oxide material	Ferro-manganese concretions, obtained from Fladbury soil series in South Nottinghamshire, were finely ground in a vibratory ball mill.
Organic matter	
Peat	Well humified basin peat from the Bush Estate, Midlothian
Humic acid	These were obtained by sequential extraction of the above peat with 0.1M $Na_4P_2O_7$ and 0.5M NaOH (Posner, 1966)
Fulvic acid	These were obtained by sequential extraction of the above peat with 0.1M $Na_4P_2O_7$ and 0.5M NaOH (Posner, 1966)

the pH with a Pye Unicam PW9418 meter and combined glass and reference electrode, calibrated with buffers of pH 4.0 and 7.0.

Mechanical analysis of soils was carried out by the pipette method of Kilmer and Alexander (1949). U.S.D.A. size limits were imposed.

Soil organic matter contents were determined by the dichromate method (Tinsley, 1950).

Methods described above and in section 3.2.2. were applied to eleven soils which were used in experimental work (table 3.3.).

Table 3.3.

Characteristics of soils used in experimental work

Soil series	Reference	Organic matter (%)	Coarse sand (%)	Fine sand (%)	Sand (%)	Silt (%)	Clay (%)	pH (in $CaCl_2$)	EDTA-extractable copper ($\mu g\ g^{-1}$)	Free manganese ($\mu g\ g^{-1}$)	Free iron ($\mu g\ g^{-1}$)
ETTRICK	1	6.66	8.24	20.48	28.72	35.76	27.29	5.1	1.47	164	16,270
LAUDER	2	3.98	17.33	25.94	43.27	33.00	17.98	5.7	2.31	359	12,250
LINHOPE	3	5.29	19.18	15.94	35.12	38.42	18.28	5.0	1.84	332	16,340
SOURHOPE	4	4.18	39.08	22.15	61.23	19.61	12.98	5.8	2.69	478	9,790
HOBKIRK	5	3.57	22.77	45.48	68.25	16.96	7.91	5.7	2.25	85	5,300
ECKFORD	6	3.19	45.18	34.76	79.94	8.81	5.47	6.5	0.41	96	4,220
ETTRICK	31	6.30	6.63	24.46	31.09	33.85	27.90	5.3	2.20	150	12,700
LAUDER	33	4.11	19.89	24.33	44.22	29.52	18.67	5.4	2.47	985	15,000
LINHOPE	35	4.47	18.80	15.54	34.34	37.85	21.10	5.2	1.77	625	19,600
SOURHOPE	45	2.55	36.67	28.25	64.92	19.10	13.67	5.4	2.36	300	11,400
HOBKIRK	49	2.59	25.23	49.15	74.38	17.77	7.37	5.7	0.91	140	4,900

3.3. Factors affecting the extractability of soil copper

Extraction with ethylenediaminetetraacetic acid (EDTA) is the most commonly used method of assessing available copper in soils. However, there is little information on whether the amount of copper extracted from a soil by EDTA remains constant or varies with changes in soil environmental conditions. If it is assumed that the method provides a reasonable measure of the availability of soil copper to plants, changes in copper availability should be reflected by changes in the amount of EDTA-extractable copper.

A study was carried out, over a period of ten months, of the effect of different soil treatments on the amount of natural and applied copper extractable with EDTA. One set of the soils used was moist incubated, in order to simulate conditions in the field, and a second set was stored dry to represent delays which often occur, in routine work, before analysis takes place.

The soils were sampled at intervals. In addition to EDTA-extractable copper, values were also obtained for isotopically exchangeable copper and the amount extracted by $CaCl_2$, two characteristics which might also be considered as estimates of available copper.

3.3.1. Preliminary studies

A collection of soils was made from 32 fields in the Scottish Borders during May and June 1977. The soils represent six series, of which three are associated with copper deficiency. Each sample, composed of about 40 cores, was air-dried and a roller mill was used

to exclude material larger than 2 mm.

The pH and EDTA-extractable copper content of each soil was determined. In addition, 4g of each soil was shaken with 40 µg copper in 40 ml 0.05M $CaCl_2$ for 24 hours. After centrifuging, the supernatant was discarded and the soil was extracted with EDTA. The amount of copper obtained in excess of the value for native copper was expressed as the percentage recovery of the copper added (table 3.4.).

The history of these soils is unknown and it is likely that, in many cases, the amounts of EDTA-extractable copper are influenced by applications of copper made over the last twenty years. Small additions are difficult to detect but it is clear that the Hobkirk sample no. 59 has been heavily supplemented. In spite, however, of any additions made in the past, a large number of the soils are still very low in copper extractable with EDTA. A third of those sampled are classified as deficient or low in copper.

The range of values obtained for the recovery of added copper is small and appears not to be related to the copper status of the soil as determined by EDTA extraction. A lower recovery might have been expected from deficient soils. The high recovery obtained (85 - 100%) was thought to reflect the short time for which the added copper had been in contact with the soil.

3.3.2. The effect of time on the recovery of added copper

The recovery experiment described above was repeated using six of the soils, with the method modified to the extent that periods of three and seven days were allowed between adding copper to the soil and re-extracting it with EDTA.

Table 3.4.

A preliminary study of 32 Borders soils

(Holkirk, Linhope and Sourhope series are associated with copper deficiency)

Soil Series	Sample No.	pH in $CaCl_2$	EDTA-extractable copper $\mu g\ ml^{-1}$	Copper deficiency classification (E.S.C.A.)	Recovery of added copper (%)
WHITSOME	28	5.7	3.67	adequate	95.1
"	43	6.0	2.20	"	92.9
"	44	5.3	1.63	"	101.8
"	47	6.0	2.05	"	94.1
"	48	5.4	0.69	deficient	92.1
HOBKIRK	29	6.0	2.44	adequate	95.4
"	36	5.2	0.87	deficient	90.6
"	37	5.3	0.40	"	94.0
"	38	5.2	0.90	"	92.4
"	42	5.2	0.91	low	89.1
"	46	5.2	1.27	"	93.9
"	49	5.7	0.91	"	93.3
"	59	5.3	11.57	adequate	100.4
LAUDER	30	5.3	3.06	"	89.3
"	33	5.4	2.47	"	91.3
"	41	4.9	1.08	low	89.0
ETTRICK	31	5.3	2.20	adequate	89.7
"	34	4.9	2.01	"	90.1
"	40	4.3	2.06	"	85.0
"	54	5.2	3.27	"	96.9
"	56	5.1	7.22	"	89.2
"	58	4.9	3.37	"	95.0
LINHOPE	32	4.9	1.16	low	85.0
"	35	5.1	1.77	adequate	87.9
"	39	5.0	1.00	low	90.7
"	53	5.4	3.14	adequate	88.3
"	55	5.1	3.18	"	96.9
"	57	4.2	3.13	"	85.5
SOURHOPE	45	5.4	2.36	adequate	89.1
"	50	5.0	0.87	deficient	89.5
"	51	5.1	3.28	adequate	86.3
"	52	6.0	1.50	"	89.2

The amount of copper extracted decreased with time to a similar extent in all soils (table 3.5.). The decrease averaged 12% after seven days.

The variation in extractability of soil copper in the absence of plant growth appears not to have been examined before. In view of the changes found to occur over a few days, a need was felt for a long-term study of the phenomenon, the results of which would be of relevance to the supplementation with copper of soils in the field.

3.3.3. A long-term incubation study

Method

Six soils, numbers 6, 31, 33, 35, 45 and 49, were chosen as representative of the range of soil series and levels of EDTA-extractable copper found in the Scottish Borders.

Into each of six 150g samples of each soil was mixed 18.75 ml of a $CuCl_2$ solution (40 μg Cu ml^{-1}, supplying 5 μg Cu g^{-1} air dry soil). A duplicate set of samples were treated with the same volume of deionised distilled water. The soils were allowed to dry and then transferred to 300 ml plastic containers with perforated lids.

Each group of six containers was divided into three pairs. One pair remained on a shelf in the laboratory. Of the other two pairs, one was wetted to 10% moisture by weight and the other to a level, previously determined for each soil, which gave the maximum water content without hindering mixing and sampling. The moisture levels ranged from 16% to 22%. The wetted soils were then placed in an incubator at 20°C.

At approximately weekly intervals the incubated tubs were made

Table 3.5.

The effect of time on the recovery of copper added to soil

Soil No.	Recovery of added copper (%)		
	After addition	After 3 days	After 7 days
28	95.1	86.9	81.9
29	95.4	90.0	85.7
30	89.3	83.3	77.3
31	89.7	78.6	77.4
32	85.0	82.3	76.7
33	89.1	88.1	79.5

up to weight with deionised distilled water, which was well mixed in. In order to obtain samples of known water content, sampling for analysis was carried out immediately after this mixing and the tubs were reweighed to obtain a new gross weight. Over a period of ten months, four determinations of EDTA-extractable copper and also three determinations of $CaCl_2$-extractable copper and isotopically exchangeable copper were carried out.

Results and discussion

Little difference was found between the results for the two moisture levels, so a mean of the data was used for a comparison of the effects of moist incubation with those of dry storage.

(a) EDTA-extractable copper

Each treatment produced a very similar trend in all six soils so that it is possible, to a considerable extent, to consider the soils together.

In both dry and moist incubated soils, with and without added copper, the amounts of EDTA-extractable copper decreased with time. The ability of EDTA to recover added copper also decreased with time (figure 3.3.). It was thought that the decrease in EDTA-extractable copper with time observed in the dry soils could have been caused by the slow recovery of the soils from the preparation for this experiment, which involved moistening and drying all soils. If this had been so, the first copper determination after the setting up of the experiment would have been expected to produce relatively high values. In fact the values obtained were no higher than those obtained previously for the same soils. Thus the soils had not been disturbed in this respect.

The decrease observed is likely to be the effect of ageing and a

Table 3.6.

Variation in EDTA-extractable copper with time

Soil		Length of time before sampling (weeks)				Units
		1(dry) 4(moist)	9	27	43	
31	dry	2.08	1.98	1.81	1.81	$\mu g\ g^{-1}$
	moist incubated	1.86	1.70	1.57	1.57	"
	dry + Cu	7.05	6.77	5.91	6.03	"
	moist incubated + Cu	6.23	5.69	4.94	4.53	"
	recovery (dry)	99.4	95.8	82.0	84.4	%
	" (incubated)	87.4	79.8	67.4	59.2	"
33	dry	2.35	2.27	2.09	2.04	$\mu g\ g^{-1}$
	moist incubated	2.28	2.12	1.87	1.95	"
	dry + Cu	6.41	6.27	5.31	5.39	"
	moist incubated + Cu	5.57	5.08	4.38	4.33	"
	recovery (dry)	81.2	80.0	64.4	67.0	%
	" (incubated)	65.8	59.2	50.2	47.6	"
35	dry	1.80	1.71	1.51	1.59	$\mu g\ g^{-1}$
	moist incubated	1.65	1.53	1.45	1.48	"
	dry + Cu	6.40	6.07	4.94	5.12	"
	moist incubated + Cu	5.22	4.73	4.13	3.75	"
	recovery (dry)	92.0	87.2	68.6	70.6	%
	" (incubated)	71.4	64.0	53.6	45.4	"
45	dry	2.00	2.03	1.85	1.81	$\mu g\ g^{-1}$
	moist incubated	1.92	1.76	1.55	1.63	"
	dry + Cu	6.66	6.68	5.53	5.76	"
	moist incubated + Cu	5.92	5.46	4.55	4.41	"
	recovery (dry)	93.2	93.0	73.6	79.0	%
	" (incubated)	80.0	74.0	60.0	55.6	"
49	dry	0.77	0.79	0.68	0.70	$\mu g\ g^{-1}$
	moist incubated	0.80	0.84	0.65	0.75	"
	dry + Cu	5.69	5.41	4.62	4.79	"
	moist incubated + Cu	4.93	4.58	3.87	3.69	"
	recovery (dry)	98.4	92.4	78.8	81.8	%
	" (incubated)	82.6	74.8	64.4	58.8	"
6	dry	0.53	0.45	0.45	0.40	$\mu g\ g^{-1}$
	moist incubated	0.46	0.45	0.39	0.46	"
	dry + Cu	5.41	5.37	4.77	4.79	"
	moist incubated + Cu	4.78	4.56	3.96	3.85	"
	recovery (dry)	97.6	98.4	86.4	87.8	%
	" (incubated)	86.4	82.2	71.4	67.8	"

Figure 3.3.

Variation in EDTA-extractable copper with time

(a) total amount (mean of six soils)

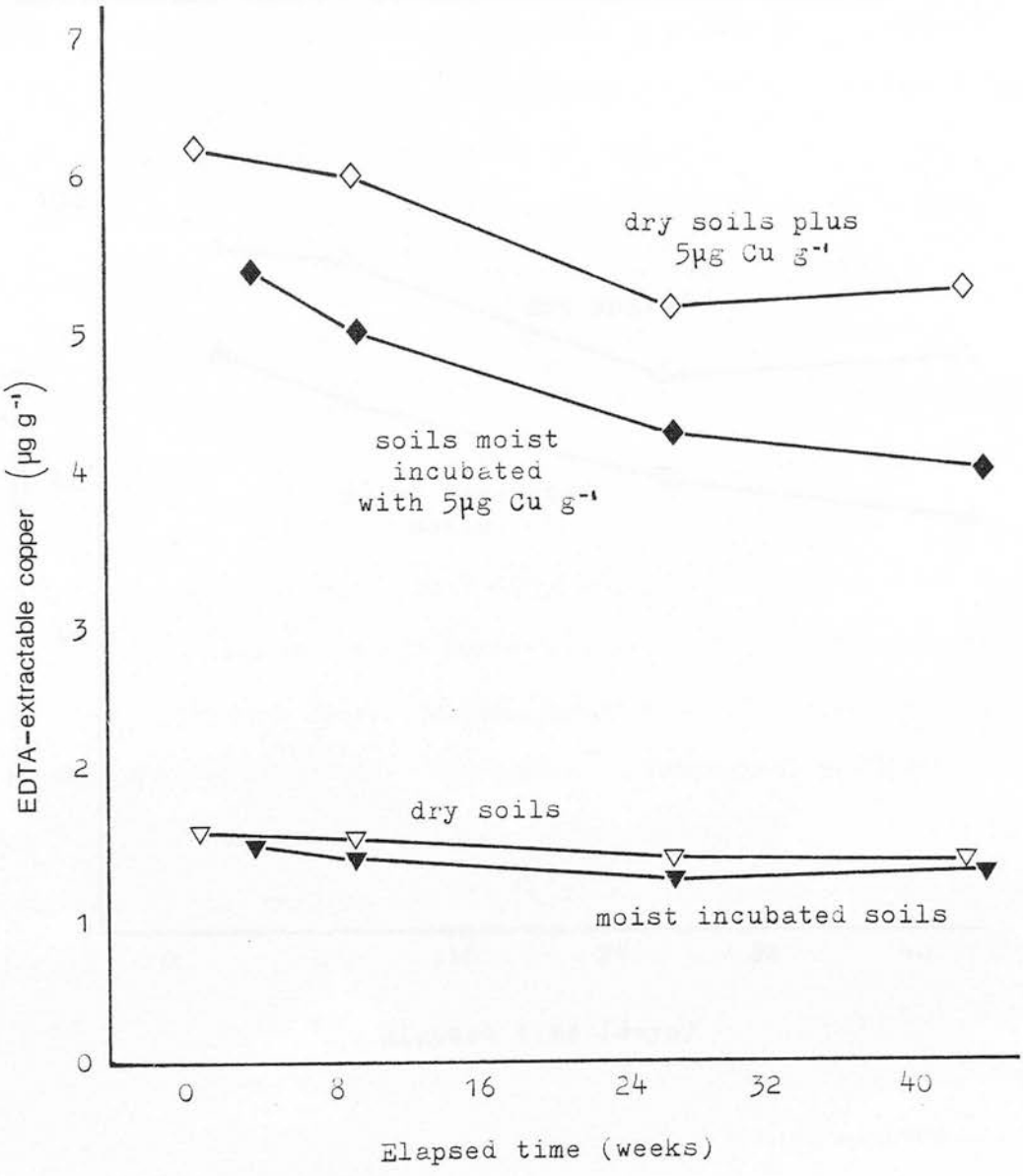
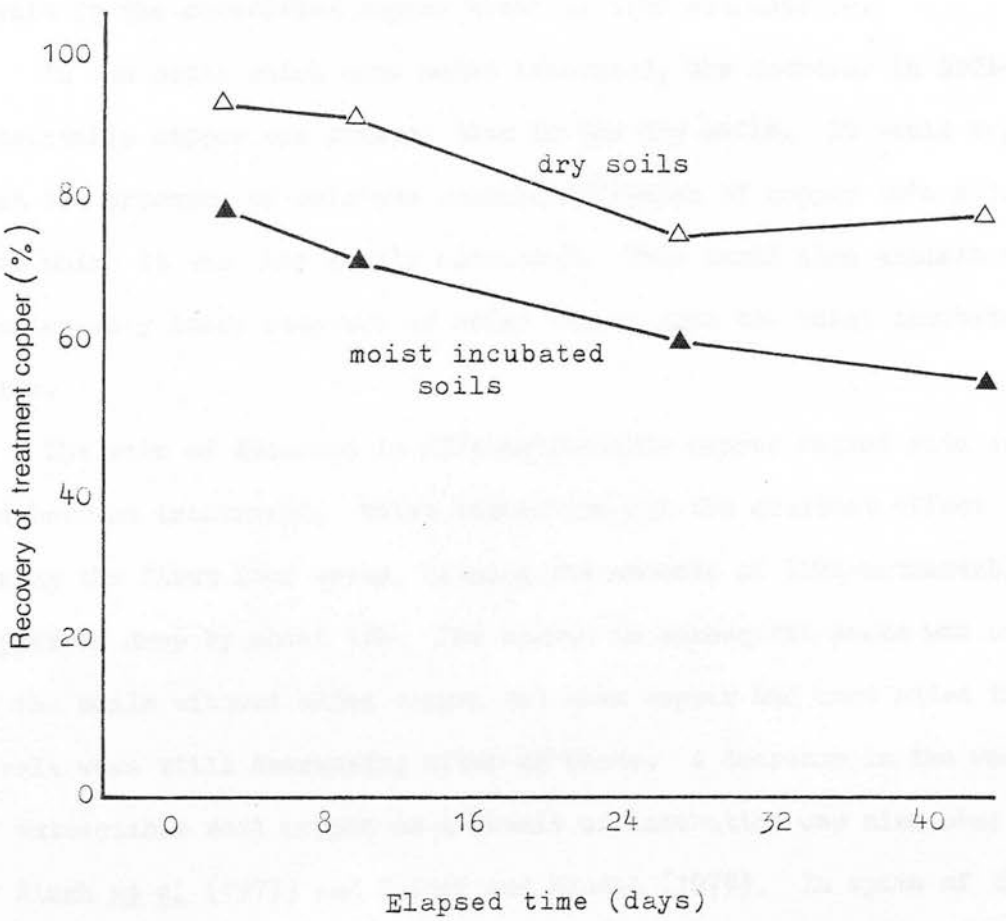


Figure 3.3.

Variation in EDTA-extractable copper with time

(b) recovery of treatment copper (mean of six soils)



possible increased degree of crystallinity of soil oxide material. A significant amount of copper is associated with oxide material in soils (McKenzie, 1967; Grimme, 1968) but Borggaard (1976), working with iron oxides, showed that only the amorphous forms are extractable with EDTA. It could well be that, of the copper in the soil oxide fraction, only that associated with amorphous oxides is extractable by EDTA. Thus any increase in crystallinity of the soil oxides would result in the associated copper becoming less extractable.

In the soils which were moist incubated, the decrease in EDTA-extractable copper was greater than in the dry soils. It would appear that the presence of moisture enabled diffusion of copper into sites from which it was less easily extracted. This would also explain the considerably lower recovery of added copper from the moist incubated soils.

The rate of decrease in EDTA-extractable copper varied with time and between treatments. Moist incubation had the greatest effect during the first four weeks, causing the amounts of EDTA-extractable copper to drop by about 10%. The change in subsequent weeks was small in the soils without added copper but when copper had been added the levels were still decreasing after 44 weeks. A decrease in the amount of extractable soil copper as a result of incubation was also observed by Singh et al (1977) and Haldar and Mandal (1979). In spite of the rate, however, at which copper became unextractable by EDTA, the shape of the graph (figure 3.3.) indicates that, under the conditions used, an application of 5 μg Cu g^{-1} to these soils would continue to provide enhanced EDTA-extractable copper levels for some years. This is confirmed by analysis of soil samples from field sites (table 3.7.).

Table 3.7.

Analysis of soils from a field site (Rumbleton Rigg)

(soils sampled June 1977)

Treatment	pH (in $CaCl_2$)	EDTA-extractable copper ($\mu g\ g^{-1}$)
No copper added	5.5	0.7
No copper added	5.5	0.8
28.0 kg $CuSo_4.5H_2O$ ha^{-1} in 1969	5.5	3.4
11.2 kg $CuSo_4.5H_2O$ ha^{-1} in 1970	5.3	1.1
11.2 kg $CuSo_4.5H_2O$ ha^{-1} in 1975	5.1	1.5

Dry soils without added copper showed a steady decrease in the amount of EDTA-extractable copper throughout the period of the experiment but there was an unexpected minimum in the values obtained from the third (October) sampling of the dry soils with added copper. The dry soils were not kept in an incubator and it is suggested that the temperature variation in the south-facing laboratory was responsible for this minimum and the higher values obtained from the fourth sampling in January. The mechanism for the increase in EDTA-extractable copper is, however, unknown.

Temperature differences may also be responsible for other differences in shape between the graphs for dry and moist incubated soils, if mobility of copper increases with temperature. Between the first and second samplings the dry soils would have been at a lower temperature than those in the incubator, whereas for the next period the dry soils may well have been warmer. If this is so, the steep parts of the two graphs for soils with added copper are associated with the warmer soils.

Even if the undulations in the graphs of copper recovery are due to temperature variation, it is still clear that the difference in the recovery of copper from dry and moist soils increased with time, indicating the importance of water content.

Recovery of added copper from individual soils was in the order $6 > 31 > 49 > 45 > 35 > 33$, a sequence bearing no apparent relationship to EDTA-extractable copper, soil pH, organic matter content or texture. However, when recovery values (the mean of four samplings) were plotted against the oxalate extractable manganese and iron contents of the soils, recovery was found to decrease as these increased. The

relationship with soil manganese was particularly clear (figure 3.4.). This was taken as evidence that soil oxides were involved in the fixation of copper in a form not extractable with EDTA, in both dry and moist soils. The two treatments differ only in the extent to which the fixation has taken place. The moister of the two treatments did not approach anaerobic conditions under which reduction of oxides would have occurred and the extra moisture content appears simply to have enabled more copper to be fixed in a non-extractable form.

McKenzie (1967) stored a suspension of a manganese oxide treated with copper for seven months. In spite of using a milder extractant, acetic acid, he too found a slight decrease in the amount of extractable copper. McLaren and Crawford (1973a) used potassium pyrophosphate in preference to EDTA for extracting copper associated with organic matter. They found that the percentage of soil copper extracted with pyrophosphate increased with the ratio of organic matter to free manganese. This suggests competition between adsorbent surfaces for copper, especially since pyrophosphate was stated to cause less solution of oxides than EDTA.

(b) $CaCl_2$-extractable copper

No trends of change with time were apparent (appendix 4) and it is likely that the values are, in fact, constant, with the variation being produced by analytical error. If this is so it is possible to reduce the errors by averaging the results from the three samplings (table 3.8.).

The soils treated with copper had twice the amount of copper extractable with $CaCl_2$ than was present in the untreated soils. This could have a considerable effect on availability.

Figure 3.4a.

Recovery of added copper from six dry stored and moist incubated soils versus soil manganese

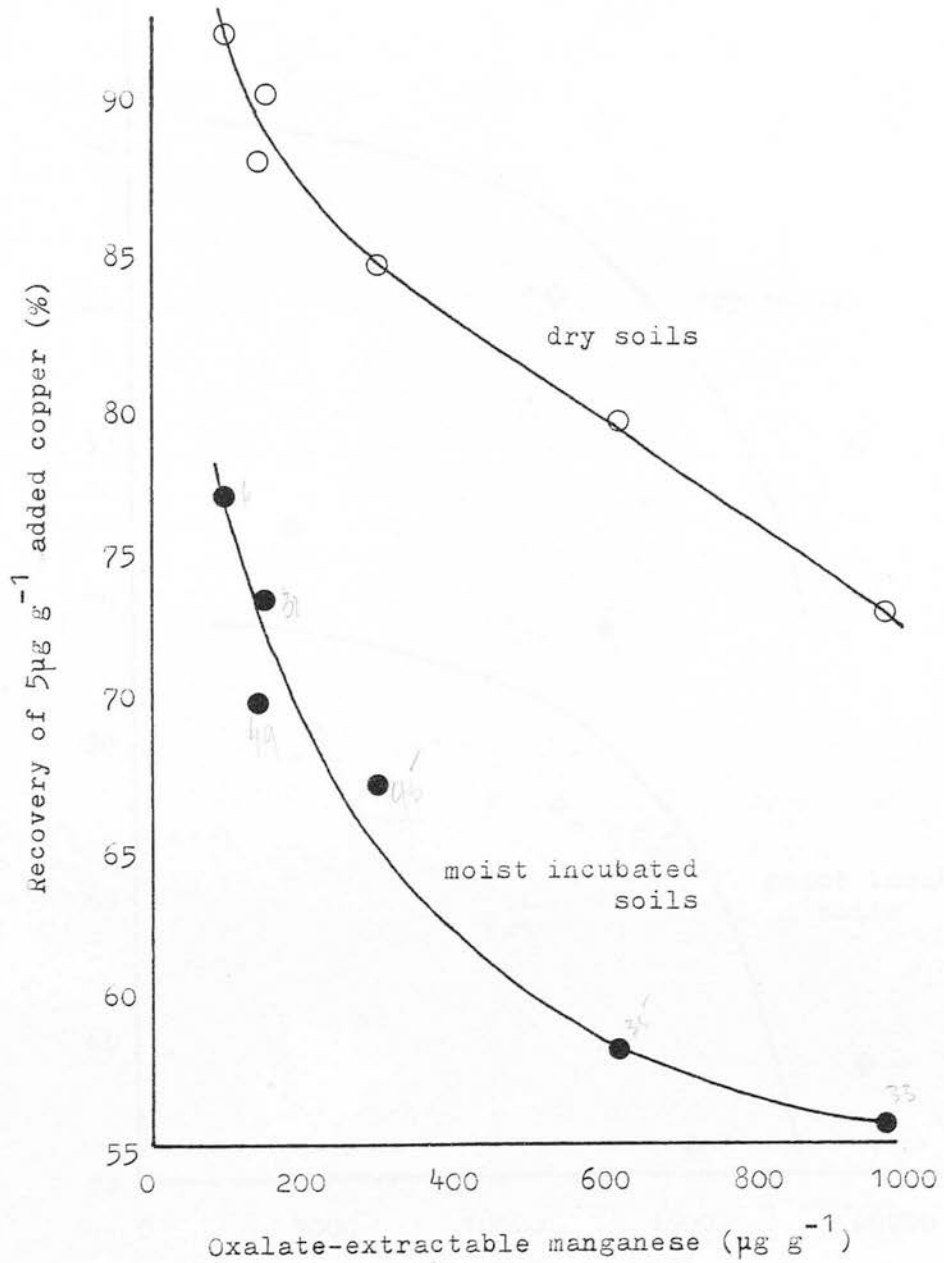


Figure 3.4b.

Recovery of added copper versus soil iron

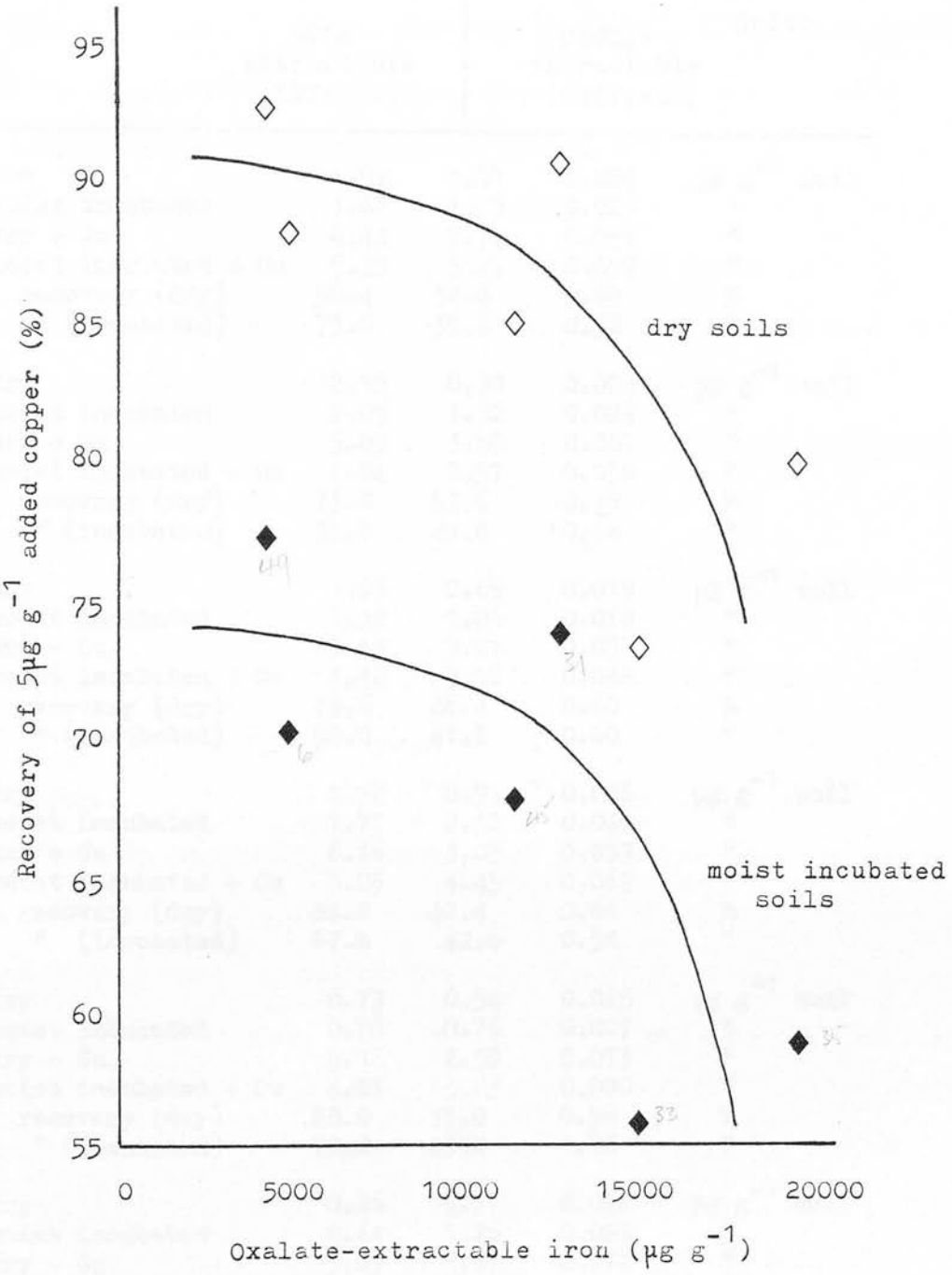


Table 3.8.

Extractable soil copper

Soil		Isotopically exchangeable (Cu-IE) EDTA-extractable (EDTA-Cu)		$CaCl_2$-extractable ($CaCl_2$-Cu)	Units
31	dry	1.92	1.01	0.024	$\mu g\ g^{-1}$ soil
	moist incubated	1.67	1.29	0.026	"
	dry + Cu	6.44	2.74	0.054	"
	moist incubated + Cu	5.35	3.27	0.052	"
	recovery (dry)	90.4	34.6	0.60	%
	" (incubated)	73.6	39.6	0.52	"
33	dry	2.19	0.90	0.024	$\mu g\ g^{-1}$ soil
	moist incubated	2.05	1.52	0.024	"
	dry + Cu	5.85	3.58	0.067	"
	moist incubated + Cu	4.84	2.97	0.056	"
	recovery (dry)	73.2	53.6	0.86	%
	" (incubated)	55.8	29.0	0.64	"
35	dry	1.65	0.69	0.019	$\mu g\ g^{-1}$ soil
	moist incubated	1.52	1.06	0.018	"
	dry + Cu	5.63	3.01	0.053	"
	moist incubated + Cu	4.46	3.12	0.048	"
	recovery (dry)	79.6	46.4	0.68	%
	" (incubated)	58.8	41.2	0.60	"
45	dry	1.92	0.91	0.026	$\mu g\ g^{-1}$ soil
	moist incubated	1.71	2.32	0.040	"
	dry + Cu	6.16	3.03	0.059	"
	moist incubated + Cu	5.09	4.45	0.069	"
	recovery (dry)	84.8	42.4	0.66	%
	" (incubated)	67.6	42.6	0.58	"
49	dry	0.73	0.54	0.025	$\mu g\ g^{-1}$ soil
	moist incubated	0.76	0.76	0.027	"
	dry + Cu	5.13	2.59	0.073	"
	moist incubated + Cu	4.27	3.23	0.080	"
	recovery (dry)	88.0	35.0	0.96	%
	" (incubated)	70.2	49.4	1.06	"
6	dry	0.46	0.77	0.028	$\mu g\ g^{-1}$ soil
	moist incubated	0.44	1.26	0.029	"
	dry + Cu	5.09	3.67	0.072	"
	moist incubated + Cu	4.29	2.82	0.051	"
	recovery (dry)	92.6	58.0	0.88	%
	" (incubated)	77.0	31.2	0.44	"

Moist incubation did not alter the levels of native copper extracted by $CaCl_2$ from those in the dry soils, indicating that the added moisture in the former had reached equilibrium with the soil. With the soils to which copper had been added, moist incubation tended to reduce the amount of copper extracted by $CaCl_2$. In the case of two of the soils, however, the opposite occurred. These two soils were those with the lowest organic matter contents, suggesting that fixation by organic matter could be responsible for the reduction in $CaCl_2$-extractable levels in the other soils. This change in distribution would not be detected by EDTA extraction, since both fixed and free forms would be extracted.

Once again the presence of moisture probably aided the diffusion of copper towards sites binding it more strongly.

(c) Isotopically exchangeable copper (Cu-IE)

No trends of change with time are visible because of the influence of the variable values for $CaCl_2$-extractable copper, used for calculating Cu-IE. The results from the three samplings were therefore averaged.

Values for Cu-IE in soils with added copper were approximately twice those for soils without added copper. The majority (80%) of this difference derives from the corresponding difference in $CaCl_2$-extractable copper values. There are, however, additional trends associated only with isotopically exchangeable copper. The effect of moist incubation on Cu-IE values of soils with added copper varied from soil to soil. Values for soils without added copper were, however, all substantially increased, by between 28% and 154%, by moist incubation. Thus the pool of native copper which is in equilibrium

with the solution phase was increased in size by moist incubation.

It is possible that, as a result of incubation, changes occur in the distribution of copper or in the nature of the sites at which it is adsorbed, which, without affecting the amount of EDTA-extractable copper, could increase the size of the isotopically exchangeable fraction.

3.3.4. Conclusions

One third of the Borders soils sampled were relatively low in copper extractable with EDTA.

In a ten-month study of six soils both with and without added copper, the amount of EDTA-extractable copper decreased with time. The decrease was larger in moist incubated soils than in those stored dry. Thus the recovery of added copper also decreased with time. All six soils responded similarly to the various treatments but the recovery with EDTA of added copper was lower in the soils with the higher oxide contents. The relationship was identical in both dry and moist incubated treatments and indicated that the non-extractability of copper was the result of its fixation by oxides.

The length of the period of moist incubation or storage did not appear to influence the amounts of copper which were extractable with $CaCl_2$ or isotopically exchangeable. Levels of copper extracted by $CaCl_2$ were doubled by the copper additions. This extractant removed similar amounts of native copper from both dry and moist incubated soils but in soils with added copper, moist incubation caused reduced amounts to be extracted, possibly because of the fixation of copper by organic matter. Although no clear trend was seen in soils to which

copper had been added, soils without added copper were found to contain larger amounts of isotopically exchangeable copper when moist incubated.

The smallest amounts of copper were measured in $CaCl_2$ extracts. Isotopic exchange and extraction with EDTA gave values of the same magnitude for soils without added copper but, of the copper added, only approximately half of that extractable with EDTA was isotopically exchangeable.

Extraction with EDTA, a well-established method of assessing available soil copper, indicated that availability is reduced by moist incubation. Although the same trend was shown by $CaCl_2$-extractable copper values for soils to which copper had been added, the only change in isotopically exchangeable copper was an increase. Because of the complexity of soil this trend is not incompatible with the others but indicates that variations in the amount of copper extractable with EDTA may not reflect what is happening in other, smaller and more available, pools. The decrease observed in EDTA values may be primarily due to a redistribution of copper from organic matter sites to those on soil oxides, or to the ageing of oxides. The significance of such a change to the plant is probably small, since only long-term availability is affected, whereas changes in isotopically exchangeable or $CaCl_2$-extractable copper might reflect short-term variations in availability.

In the field, the decrease found to occur in EDTA-extractable copper is unlikely to continue indefinitely, since it has been shown that trace elements, including copper, are mobilised by waterlogging (Beckwith *et al*, 1975; Sims and Patrick, 1978). Thus a cycle of decrease and increase in availability is possible. The changes observed

in dry soils indicate that, where soil samples are stored for long periods of time in the laboratory, values from analysis will require careful interpretation.

3.4. The distribution of copper within soils

3.4.1. The effect of cultivation

As well as reducing the total soil copper level by removal of the element in crops, continuous cultivation and cropping are thought to modify the proportions in which different forms of copper in the soil are present and thus affect its availability to plants. Grimme (1967a) showed, by fractionation, differences in copper distribution between podzolic soils under forest and those under arable cultivation. In order to investigate the matter further, pairs of samples of soils were obtained from farms where adjacent areas were under continuous cultivation and permanent pasture. A modified form (appendix 1) of the fractionation scheme of McLaren and Crawford (1973a) was used to compare the distribution of copper within these pairs (table 3.9a.).

Results and discussion

Although the two samples of each pair of soils necessarily came from sites which are spatially separated, mechanical analysis indicates that they are very similar. Reduced organic matter levels in the arable soils demonstrate the effect of continuous cultivation but the amounts of EDTA-extractable copper do not show a clear trend.

There are no large differences in total copper content between the members of each pair of soils and although in four pairs the arable values are the lower, the opposite is true for the remainder. The two treatments were not expected to differ significantly in this respect, as the arable soils sampled are unlikely to have been under continuous cultivation for more than 20 years. During that time, 0.04 $\mu g\ Cu\ g^{-1}$ would have been removed, at most, per year and there would

Table 3.9a.

Forms of copper in pasture and arable soils

($\mu g\ Cu\ g^{-1}$ soil)

Soil series		Soluble plus exchangeable copper	Weakly bound specifically adsorbed copper	Organic copper	Oxide occluded copper	Residual copper	Total copper
RAGDALE	PASTURE	0.047	0.100	4.74	2.55	10.32	15.96
	ARABLE	0.024	0.070	0.96	2.45	8.36	13.68
STIRLING	PASTURE	0.078	0.140	6.23	1.78	12.79	20.20
	ARABLE	0.035	0.130	7.46	2.24	11.80	23.43
HUMBIE	PASTURE	0.106	0.330	5.96	2.64	9.26	19.50
	ARABLE	0.063	0.185	5.15	5.19	8.90	21.47
DENCHWORTH	PASTURE	0.079	0.115	3.11	6.94	10.50	25.55
	ARABLE	0.036	0.070	3.51	3.62	9.83	20.36
POW	PASTURE	0.077	0.205	6.87	6.59	15.82	29.51
	ARABLE	0.065	0.295	5.92	3.84	13.47	25.78
BIEL	PASTURE	0.144	0.185	6.42	9.05	12.64	27.16
	ARABLE	0.046	0.220	5.73	4.00	11.00	22.71
KILMARNOCK	PASTURE	0.067	0.095	3.99	7.66	8.47	22.59
	ARABLE	0.050	0.167	5.18	7.58	10.13	19.64

Table 3.9b.

Properties of pasture and arable soils

Soil series		Organic matter (%)	Sand (%)	Silt (%)	Clay (%)	Free Iron (%)	Free manganese ($\mu g\ g^{-1}$)	EDTA-extractable copper ($\mu g\ g^{-1}$)
RAGDALE	PASTURE	3.92	38	15	45	1.54	60	5.4
	ARABLE	1.33	53	12	35	1.22	70	3.6
STIRLING	PASTURE	5.56	11	61	28	2.60	90	3.7
	ARABLE	2.14	13	55	32	1.72	70	5.1
HUMBIE	PASTURE	3.35	56	15	29	1.52	200	5.8
	ARABLE	2.35	54	18	28	3.00	650	6.1
DENCHWORTH	PASTURE	5.83	30	28	42	1.26	120	6.5
	ARABLE	2.15	34	22	44	2.20	120	3.9
POW	PASTURE	4.35	45	35	20	1.75	320	8.1
	ARABLE	1.92	41	37	22	2.05	290	7.7
BIEL	PASTURE	3.40	58	24	18	240	420	7.6
	ARABLE	1.96	47	30	23	2.50	540	6.7
KILMARNOCK	PASTURE	4.64	51	23	26	1.66	280	4.5
	ARABLE	2.40	51	22	27	1.60	220	6.4

have been small losses too from the pasture soils.

Residual copper levels also are similar in both arable and pasture soils. Since this is an extremely immobile copper fraction, this was not unexpected. Differences were expected, however, in the more available fractions, since all the arable soils have considerably reduced organic matter contents and cultivation is likely to have caused changes in the nature of the oxide fractions. Thus it was surprising to find that, overall, there had been no significant redistribution of copper between the organic, oxide and weakly bound specifically adsorbed fractions.

Grimme (1967a) also found that the silicate-bound and occluded fractions of copper did not vary between forest and arable soils but, for some reason, the organically-bound fractions were smaller in soils under forest. Also, Krahmer and Bergman (1978) found that the fraction of copper associated with organic matter and extractable with NaOH + EDTA was smaller in pasture than in arable soils. However, Beckwith et al (1975) found that EDTA extracted a larger amount of copper from pasture soils.

One would expect that the higher organic matter levels in the pasture soils would adsorb more copper and thus decrease the amount which was soluble. Levels of ionic copper in solution are probably affected in this way, since soil solutions from woodland soils have been found to contain less ionic copper than solutions from arable soils (Hodgson et al, 1965). However, measurements of soluble and exchangeable copper in arable and pasture soils showed the opposite trend, with the higher value for each pair of soils being associated with the pasture sample. Determination of the optical density of the

same extracts revealed that, with each pair of samples, the higher value was associated with pasture soil (figure 3.5.). This was taken as indicating that the pasture soils contained larger amounts of soluble organic matter. Thus, the higher soluble copper contents of the pasture soil extracts could be the result of a greater degree of organic complexing of copper.

3.4.2. Determining the extent of organic complexing of copper in solution

The example described above is not the first to indicate that the complexing of copper, by soluble organic matter, results in total copper levels in solution that are considerably higher than the simple ionic Cu^{2+} concentration of the system at equilibrium. The presence of complexed copper in solution is likely to result in increased availability of solution copper, although the uptake preferences of the plant are not clear. Most methods of determining copper levels in solution measure the total present, yet information on the relative abundance of the complexed and ionic forms in soil solution would clarify their roles. With this in mind, two possible methods of determining the extent of complexing in natural systems were investigated.

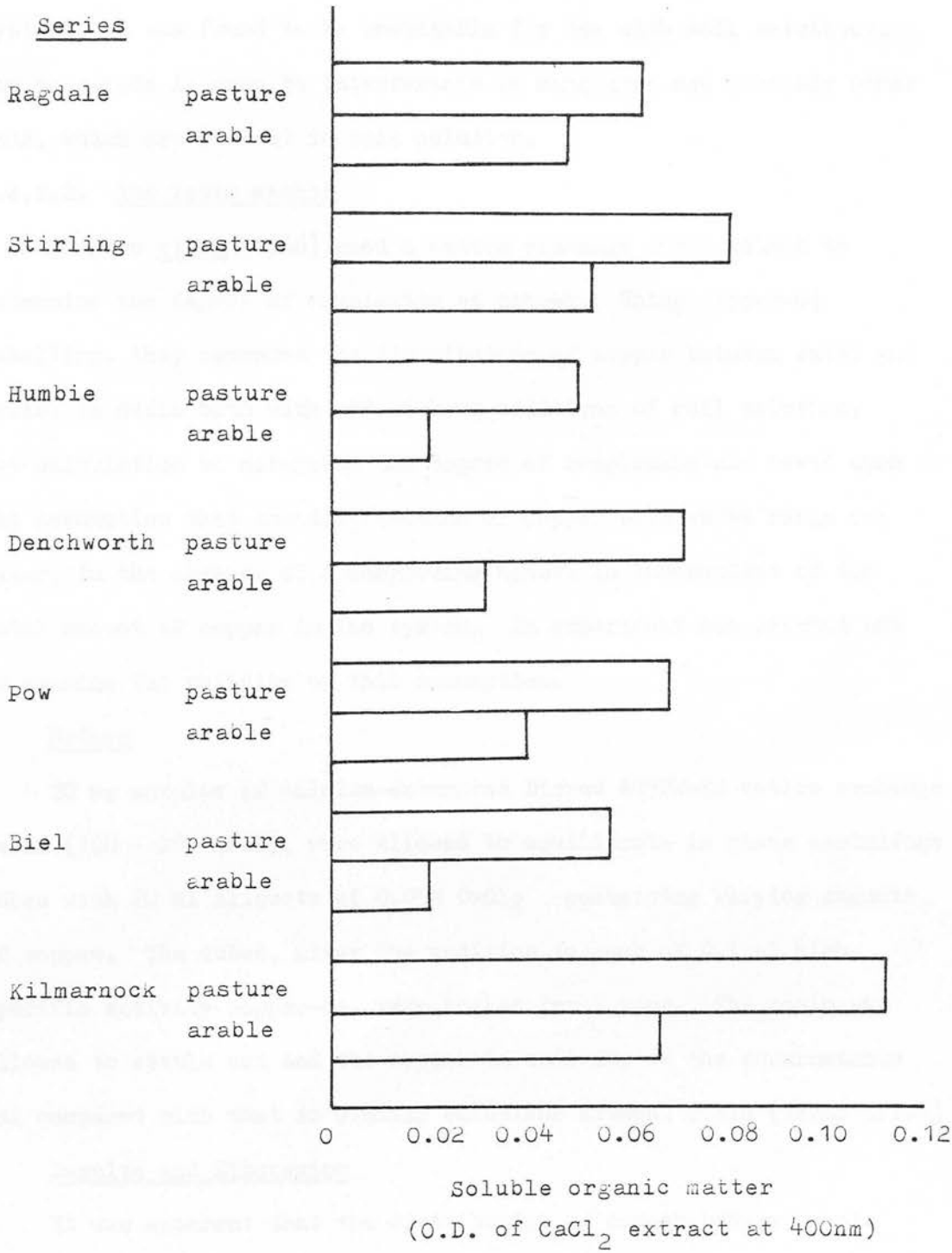
3.4.2.1. The cupric ion electrode

A combination of measurements of the level of Cu^{2+} in solution, using a specific ion electrode, and determination of total copper by atomic absorption spectrophotometry, would give indirectly a value for the amount of complexed copper in a solution.

Soil solution samples were extracted by centrifuging (3,000 rpm

Figure 3.5.

Soluble organic matter in pasture and arable soils



for 25 minutes) soil samples which had been maintained at field capacity for one week. Total copper concentrations in these samples varied from 0.019 to 0.101 $\mu g\ ml^{-1}$. Although the cupric ion electrode was used successfully to determine Cu^{2+} concentrations in artificial systems, it was found to be unsuitable for use with soil solutions. The electrode is open to interference by manganese and possibly other ions, which are present in soil solution.

3.4.2.2. The resin method

Hodgson et al (1966) used a cation exchange resin method to determine the degree of complexing of copper. Using copper-64 labelling, they measured the distribution of copper between water and resin, in media both with and without additions of soil solution. The calculation to determine the degree of complexing was based upon the assumption that the distribution of copper between the resin and water, in the absence of a complexing agent, is independent of the total amount of copper in the system. An experiment was carried out to examine the validity of this assumption.

Method

20 mg samples of calcium-saturated Biorad AG50W-X4 cation exchange resin (100 - 200 mesh), were allowed to equilibrate in glass centrifuge tubes with 20 ml aliquots of 0.05M $CaCl_2$ containing varying amounts of copper. The tubes, after the addition to each of 0.1 ml high specific activity copper-64, were shaken for 1 hour. The resin was allowed to settle out and the copper-64 activity of the supernatants was compared with that in similar solutions without resin (table 3.10.)

Results and Discussion

It was apparent that the distribution of copper between resin

Table 3.10.

Distribution of copper between 0.05M $CaCl_2$ and Biorad AG 50W-XL cation exchange resin

Initial solution copper concentration ($\mu g\ ml^{-1}$)	Copper adsorbed by resin (%)	
	Copper-64 added after copper	Copper-64 added after copper
0.1	6.0	11.0
0.01	9.8	29.0
0.001	12.6	-
0.0001	14.0	-

and solution is affected by the amount of copper in the system. In addition, when the experiment was repeated with the copper and copper-64 added at the same time, different values again were obtained for the distribution. It appears that some of the copper adsorbed by the resin is not isotopically exchangeable, especially at low site coverage. Thus the accuracy of the method, and thus its value, is considerably reduced.

3.4.3. Conclusions

The differences in management of permanent pasture and continuous cultivation have not yet had a significant effect on the total and residual soil copper contents. Surprisingly, there had been no redistribution of copper between organic, oxide and weakly bound specifically adsorbed fraction, in spite of organic matter levels having been halved by arable cultivation. The pasture soils all had higher levels of soluble copper than those under cultivation, apparently as a result of solution levels being enhanced by soluble organic complexes of copper.

Of the two methods for estimating the extent of this complexing that were investigated, neither could be satisfactorily applied to soil solution.

3.5. The interaction of copper with soil materials at low solution concentrations of copper

From the literature it appears that the levels of ionic copper in solution are very low and are regulated by adsorption/desorption phenomena associated with the colloidal soil materials (McLaren and Crawford, 1973a; Quirk and Posner, 1975; Fassbender and Seekamp, 1976). Although copper complexed by organic matter in solution supplements the ionic copper to a considerable extent (Hodgson et al, 1965, 1966) it is apparent that, in almost every study of the adsorption of copper by soil materials, the large amount of the element added has resulted in a level of copper in the solution phase of the system at equilibrium far higher than those measured in natural soil solution. This is important because there is evidence that the interaction of soil and copper differs according to the amount of copper involved (Plessis and Burger, 1971). Thus only adsorption studies resulting in equilibrium solution concentrations of copper within the range found in natural soil solutions will be of direct relevance to plant availability problems.

The difficulty in interpreting the results of the incubation studies reinforced the need for individual studies of each of the soil components known to retain copper. The experiments described below were designed to provide information about the reactions of copper with different soil materials, at copper concentrations similar to those found in natural soil solution.

3.5.1. The adsorption of copper by soil materials

3.5.1.1. Method

In order to determine the low solution copper concentrations after equilibration more accurately than is possible by spectrophotometric methods, the isotope copper-64 was used as a tracer.

Duplicate samples of adsorbent material (0.1g) were equilibrated in 15 ml polypropylene centrifuge tubes with 10 ml aliquots of solutions containing known amounts of copper labelled with about 50 μCi of the isotope copper-64. Except where stated, all solutions were 0.05M with respect to $CaCl_2$. The pH of samples was adjusted, normally to a value of 6.0, with small amounts of 0.1N HCl or saturated $Ca(OH)_2$ solution. Following equilibration at 20°C on an end-over-end shaker, the samples were centrifuged for 15 minutes at 6,800 rpm and the proportion of copper-64 activity remaining in the supernatant determined. The amount of copper adsorbed was calculated from this and the copper concentration in the reference solution, which was determined by atomic absorption spectrophotometry.

3.5.1.2. Time study of adsorption

Preliminary experiments were carried out to examine the rate at which adsorption occurred and to determine an appropriate duration for later studies. For these, however, larger tubes were used and the contents increased sevenfold to enable several solution samples to be removed for determining copper-64 activity without large changes in the ratio of solution to solid. The use of higher specific activity copper-64 (30 - 70 μCi $tube^{-1}$) allowed extension of the time study over a longer period. The 100 ml polypropylene tubes used were removed from the rotary shaker at varying intervals and centrifuged.

A sample of supernatant (1 ml) was removed before returning the tubes to the shaker.

The results (table 3.11., figure 3.6.) show the decrease in solution copper-activity with time during the adsorption of copper by humic acid, soil oxide and montmorillonite at pH 6.0. The curves for adsorption by humic acid and soil oxide are virtually identical and are plotted as one. Most of the adsorption by these two materials took place during the first hour, with no significant removal of copper from solution after the tenth hour. Montmorillonite adsorbed the bulk of the solution copper fairly rapidly but the adsorption continued over a much longer period of time.

Although Forbes et al (1976) found adsorption by goethite to be rapid, McKenzie (1972), working with manganese oxide, obtained a continuing small amount of adsorption after the rapid initial phase which was not found in this experiment with soil oxide material. Cavallaro and McBride (1978), working with soils, obtained a similar prolonging of adsorption, which could be attributed to the clay fraction.

The time which could be allowed for equilibration was restricted by the short half-life (12.8 hours) of the copper-64 isotope. For accurate results the shortest equilibration period had to be balanced against the degree of equilibrium achieved. A period of 16 hours was chosen, during which adsorption in the oxide and organic matter systems was completed. In other systems, adsorption was over 90% of the total.

3.5.1.3. The influence of pH on adsorption

Previous studies, for example by McLaren and Crawford (1973b),

Table 3.11.

Time study of adsorption of copper by soil materials

Time (hours)	Humic acid		Soil oxide		Montmorillonite	
	copper in soln. (μg ml^{-1})	% Cu-64 remaining in soln.	copper in soln. (μg ml^{-1})	% Cu-64 remaining in soln.	copper in soln. (μg ml^{-1})	% Cu-64 remaining in soln.
½	0.055	2.74	0.085	4.97	-	-
1	0.042	2.09	0.042	2.47	-	-
2	0.031	1.57	0.021	1.22	-	-
4	0.037	1.33	0.013	0.77	-	-
8	0.021	1.07	0.009	0.57	-	-
14	0.023	0.83	0.020	0.69	0.147	10.28
24	0.021	1.03	0.008	0.47	-	-
34½	0.021	0.75	0.012	0.41	0.055	3.82
48½	0.019	0.97	0.008	0.46	-	-
62	0.019	0.68	0.016	0.56	0.029	2.06
88	0.021	0.73	0.018	0.63	0.025	1.74

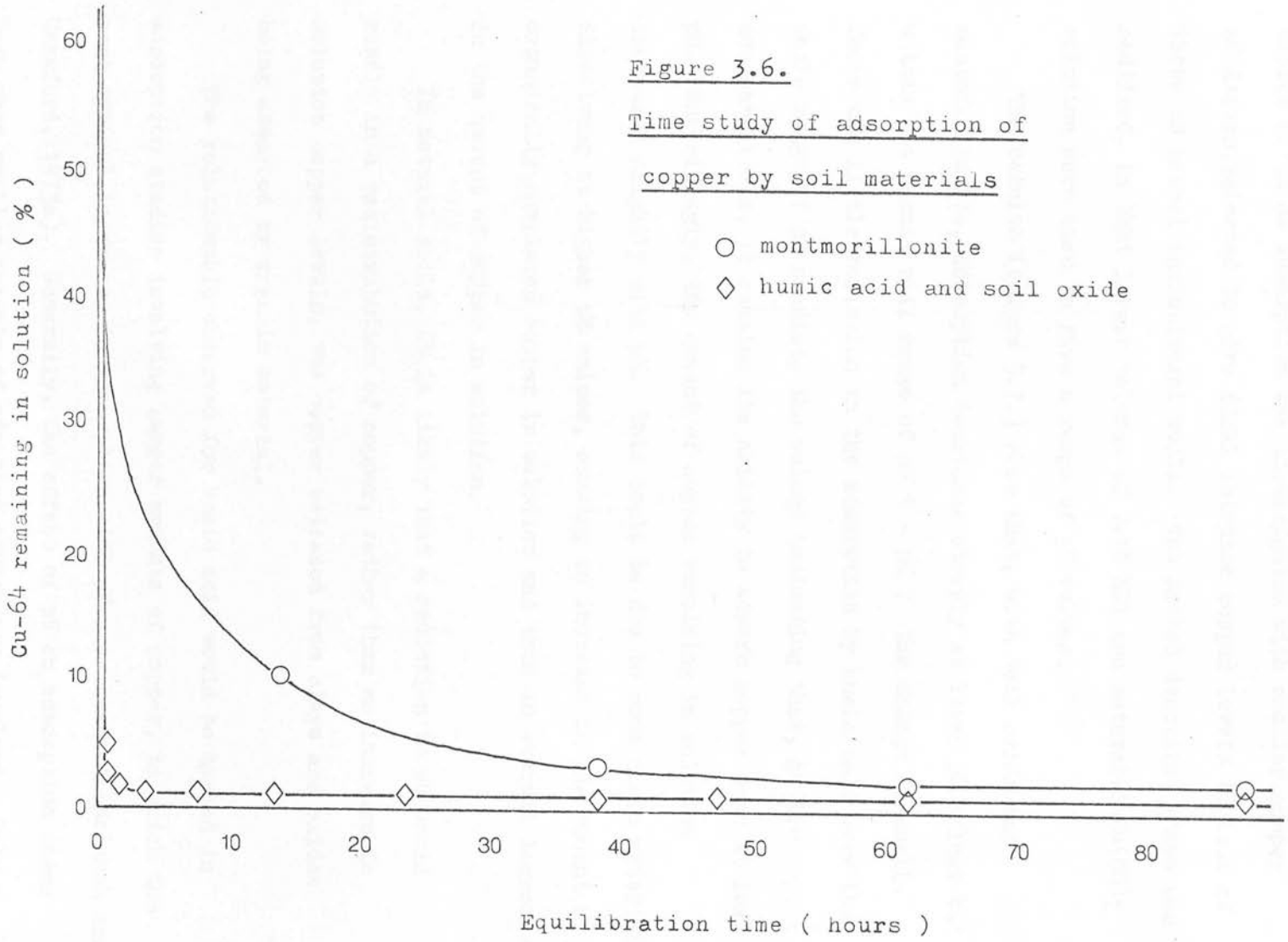


Figure 3.6.

Time study of adsorption of copper by soil materials

○ montmorillonite

◇ humic acid and soil oxide

indicate that, at higher concentrations of copper, its adsorption by soil materials is considerably influenced by pH. The extent of the effect of pH on adsorption was investigated with smaller copper additions, selected to give final solution copper levels typical of those in normal agricultural soils. The method described above was modified, in that larger volumes of 0.1N HCl and saturated $Ca(OH)_2$ solution were used to give a range of pH values.

The results (figure 3.7.) show that, with soil oxide and montmorillonite, adsorption decreases steeply at lower pH values but within the normal soil range of pH 5 - pH 7 the change is small. There was little variation in the adsorption by humic acid over the whole range of pH studied, the values indicating that, at low copper concentrations, it retains its ability to adsorb copper even at low pH. Surprisingly, the amount of copper remaining in solution increased slightly with pH. This could be due to some humic material dissolving at higher pH values, causing an increase in the amount of organically complexed copper in solution and thus an overall increase in the amount of copper in solution.

In natural soils, it is likely that a reduction in pH would result in a redistribution of copper, rather than an increase in solution copper levels, the copper released from clays and oxides being adsorbed by organic material.

The relationship observed for humic acid would be masked in adsorption studies involving larger amounts of copper, in which the more typical increases in adsorption with pH are obtained (McLaren and Crawford, 1973a). Generally, the effect of pH on adsorption seems less when smaller amounts of adsorbed copper are involved. Only a

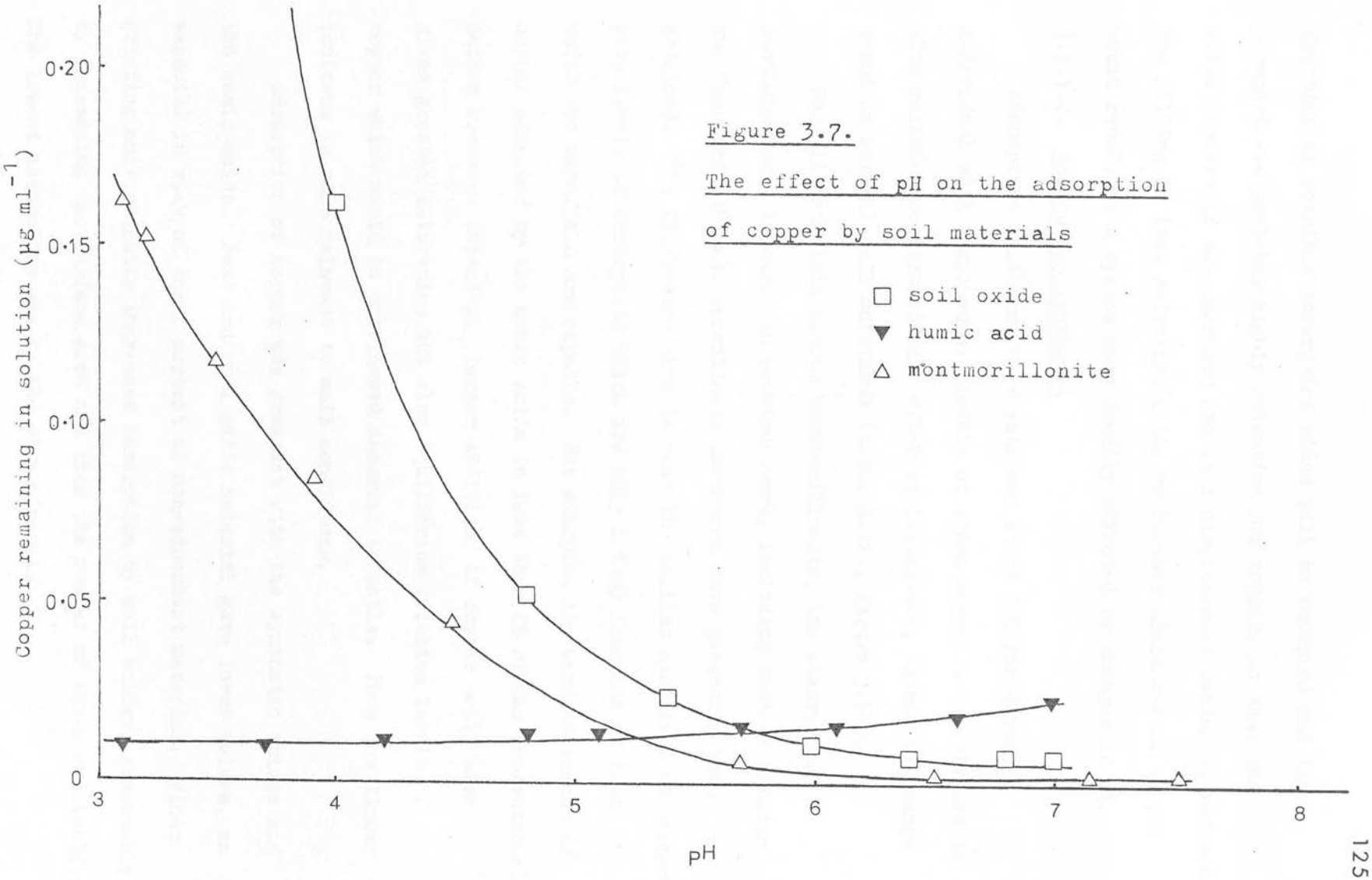


Figure 3.7.

The effect of pH on the adsorption of copper by soil materials

fraction of possible adsorption sites will be occupied and those occupied are probably highly selective for copper, so that the effectiveness of the hydrogen ion as a displacement cation is reduced. The filling of less selective sites by further additions of copper would result in a system more readily affected by changes in pH.

3.5.1.4. Adsorption isotherms

Adsorption isotherms were obtained at pH 6.0 for eleven individual soil components. Levels of added copper were selected to give solution concentrations, after equilibration, within the range found in natural soil solutions (table 3.12., figure 3.8.).

For all materials except montmorillonite, the adsorption isotherms were linear. In previous work, including that of McLaren and Crawford (1973a), curvilinear isotherms have generally been obtained. The difference here is that the smaller additions of copper gave levels of adsorption which are only a tiny fraction of that which the materials are capable. For example, the maximum amount of copper adsorbed by the humic acids is less than 2% of the conventional cation exchange capacity. Larger additions of copper would have given greater saturation but also equilibrium solution levels of copper which would be considered abnormal in soils. Thus the linear isotherm is more relevant to soil conditions.

Adsorption of copper was greatest with the synthetic oxides and the humic acids. Peat and soil oxide material gave lower values, as expected in view of their content of non-adsorbent material. Finer grinding only slightly increased adsorption by soil oxides, presumably by increasing the surface area and thus the number of sites available. The lowest adsorption was by the clay minerals.

Table 3.12a.

Adsorption of copper by peat and individual organic matter fractions at pH 6.0

Material	Initial Soln. Cu Conc. ($\mu g\ ml^{-1}$)	Equilibrium Soln. Cu Conc. ($\mu g\ ml^{-1}$)	Cu adsorbed by material ($\mu g\ g^{-1}$)
Peat	1.48	0.011	160.8
	2.15	0.015	217.7
	3.25	0.020	357.7
	5.75	0.037	626.8
	10.80	0.063	1159.0
Humic acid (pyrophosphate extraction)	1.48	0.007	168.6
	2.15	0.010	240.6
	3.25	0.012	364.3
	5.75	0.020	649.6
	10.80	0.036	1264.2
Fulvic acid (pyrophosphate extraction)	1.48	0.025	154.7
	2.15	0.037	227.1
	3.25	0.052	352.7
	5.75	0.093	657.5
	10.80	0.161	1162.5
Humic acid (sodium hydroxide extraction)	1.48	0.004	159.7
	2.15	0.006	235.1
	3.25	0.007	361.9
	5.75	0.015	634.6
	10.80	0.025	1235.0
Fulvic acid (sodium hydroxide extraction)	1.48	0.031	162.5
	2.15	0.040	237.5
	3.25	0.054	365.7
	5.75	0.108	650.8
	10.80	0.202	1199.9

Table 3.12b.

Adsorption of copper by soil colloidal material at pH 6.0

Material	Initial Soln. Cu Conc. ($\mu g\ ml^{-1}$)	Equilibrium Soln. Cu Conc. ($\mu g\ ml^{-1}$)	Cu adsorbed by material ($\mu g\ g^{-1}$)
Iron oxide (artificial)	0.90	0.002	89.5
	1.85	0.003	184.3
	2.67	0.004	267.7
	4.65	0.009	462.5
	9.55	0.020	950.6
Manganese oxide (artificial)	0.90	0.003	89.2
	1.85	0.005	183.5
	2.67	0.006	266.8
	4.65	0.006	466.0
	9.55	0.016	949.6
Soil oxide material	0.45	0.004	44.5
	0.65	0.004	64.9
	0.90	0.006	88.9
	1.85	0.012	184.4
	2.67	0.019	265.1
Soil oxide material (finely ground)	0.45	0.002	44.6
	0.65	0.003	64.3
	0.90	0.003	90.0
	1.85	0.006	184.8
	1.67	0.008	266.7
Illite	0.977	0.230	74.7
	0.496	0.113	38.3
	0.311	0.067	27.7
	0.167	0.033	13.4
	0.095	0.019	7.6
Kaolinite (pH 5.4)	0.977	0.777	20.0
	0.496	0.361	13.5
	0.311	0.213	9.8
	0.167	0.109	5.8
	0.095	0.058	3.7
Montmorillonite	0.13	0.001	12.9
	0.16	0.002	15.9
	0.24	0.002	23.8
	0.43	0.005	42.3
	0.90	0.017	89.0
	1.83	0.080	175.0
	2.77	0.210	256.6
	4.61	0.583	403.1
	9.27	2.081	716.4

Figure 3.8.

Copper adsorption isotherms for soil materials

(a) organic materials

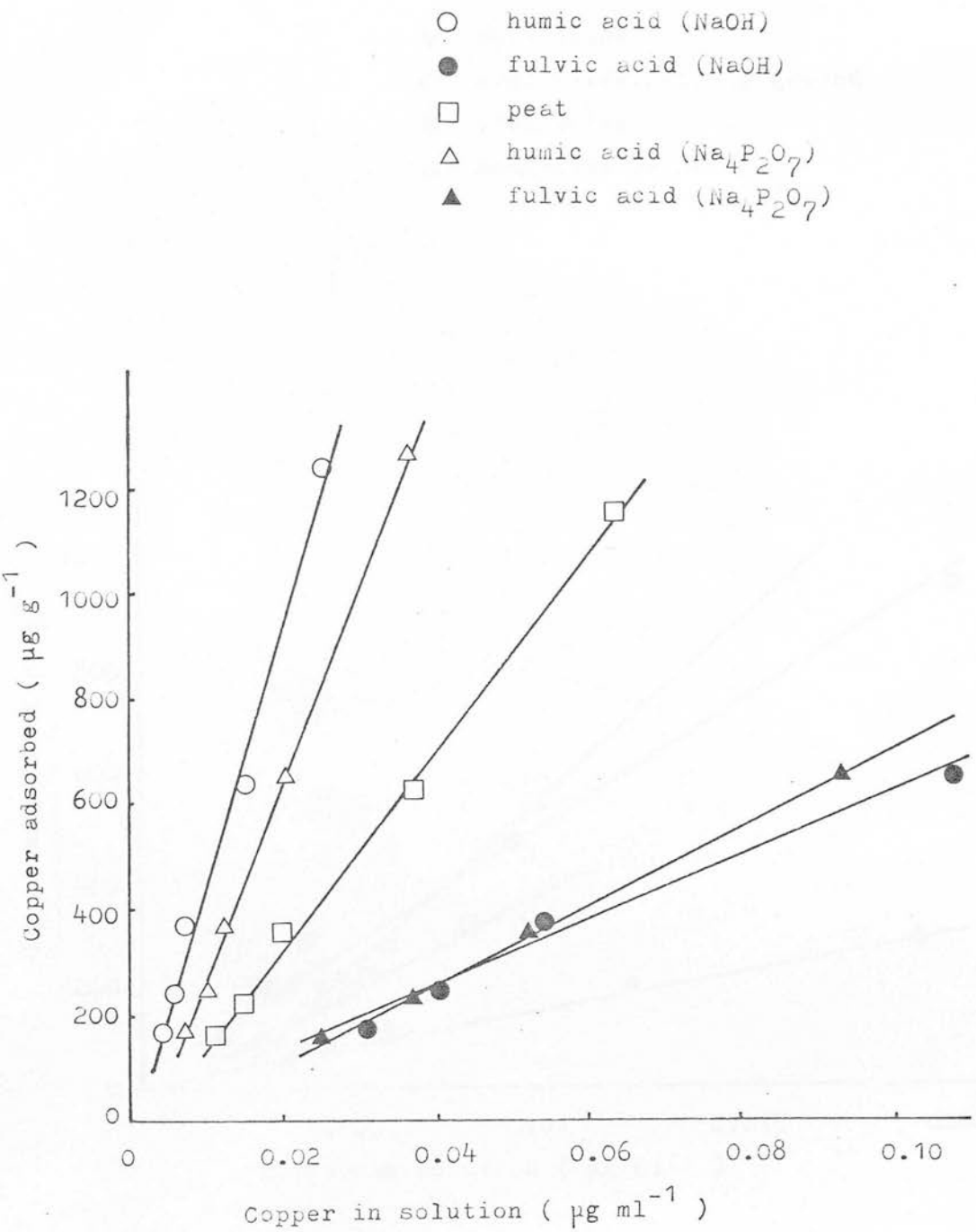


Figure 3.8.

Copper adsorption isotherms for soil materials

(b) oxides

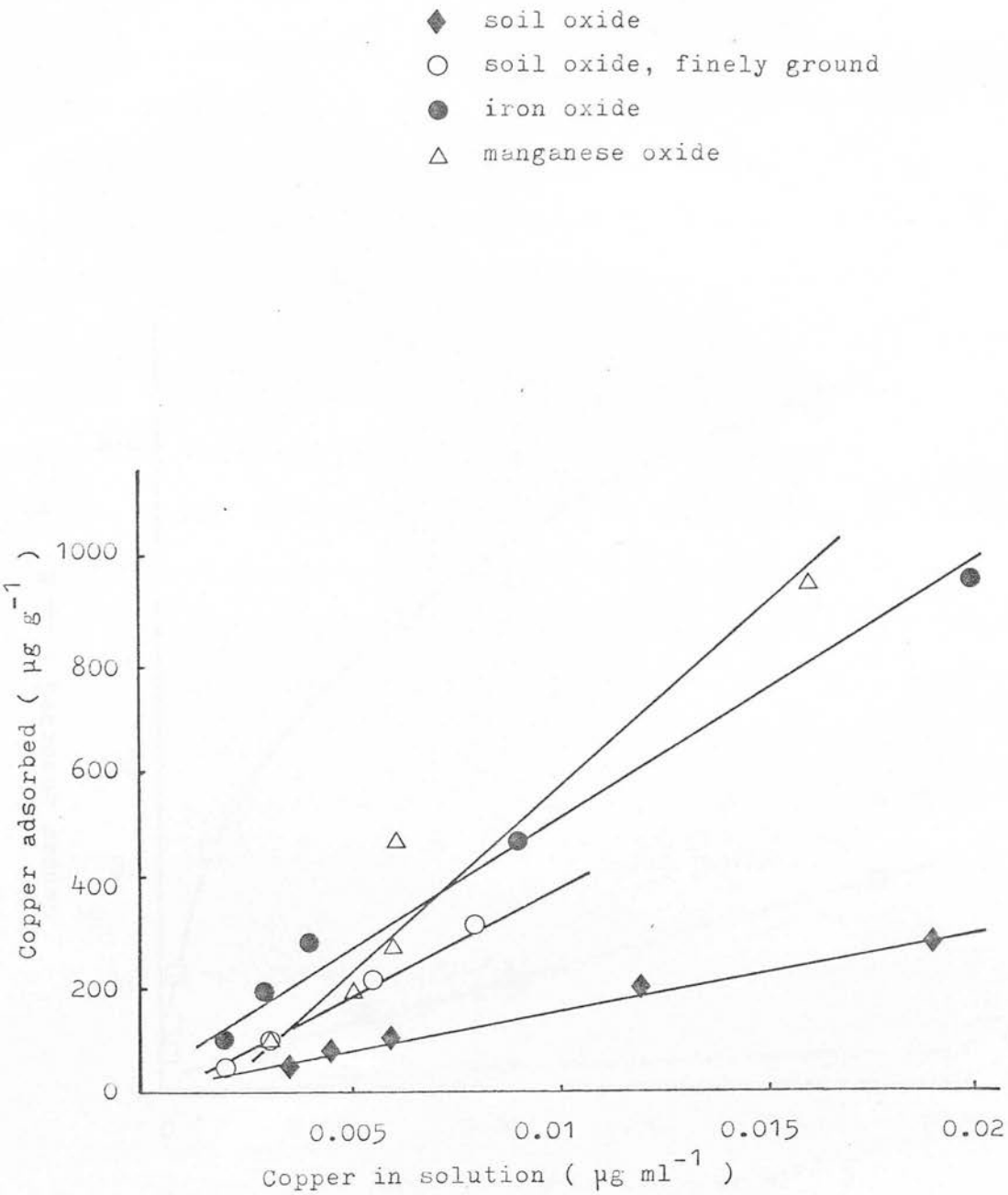
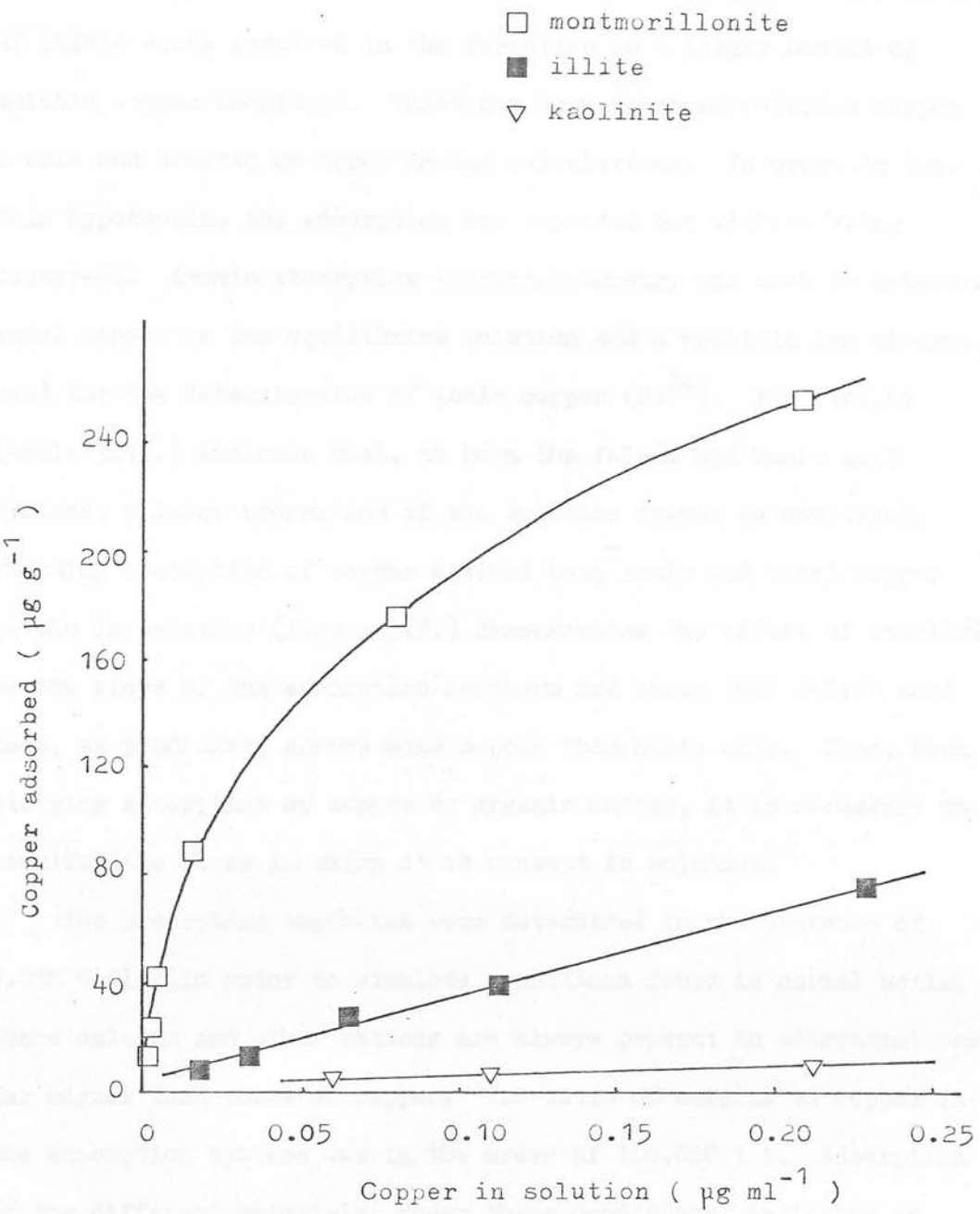


Figure 3.8.

Copper adsorption isotherms for soil materials

(c) clay minerals



It might have been predicted, from the greater number of functional groups associated with the more oxidised fulvic material, that it would adsorb more copper than the humic acid. The opposite occurred. A possible reason for this is that the greater solubility of fulvic acids resulted in the formation of a larger amount of soluble copper complexes. These may have enhanced solution copper levels and created an error in the calculations. In order to test this hypothesis, the adsorption was repeated but without using copper-64. Atomic absorption spectrophotometry was used to determine total copper in the equilibrium solution and a specific ion electrode used for the determination of ionic copper (Cu^{2+}). The results (table 3.13.) indicate that, in both the fulvic and humic acid systems, a large proportion of the solution copper is complexed. Plotting adsorption of copper against both ionic and total copper levels in solution (figure 3.9.) demonstrates the effect of complexing on the slope of the adsorption isotherm and shows that fulvic acid does, as predicted, adsorb more copper than humic acid. Thus, when studying adsorption of copper by organic matter, it is necessary to consider the forms in which it is present in solution.

The adsorption isotherms were determined in the presence of 0.05M $CaCl_2$, in order to simulate conditions found in normal soils, where calcium and other cations are always present in concentrations far higher than those of copper. The ratio of calcium to copper in the adsorption systems was in the order of 100,000 : 1. Adsorption by the different materials, under these conditions, indicates an extremely high degree of selectivity for copper. The degree of selectivity varies between individual materials, as is indicated by

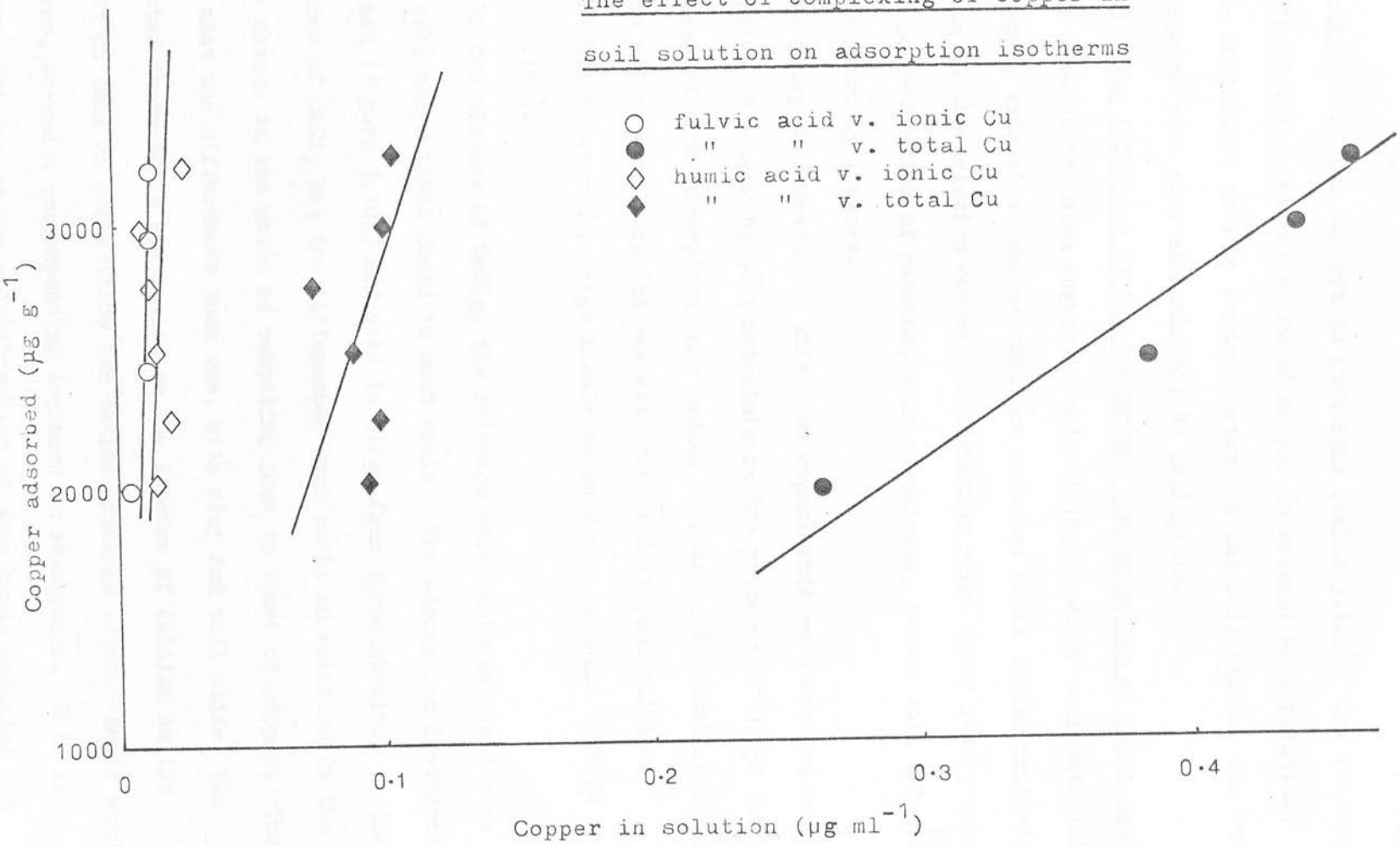
Table 3.13.

Complexing of copper in solutions in equilibrium with humic and fulvic acids

Material	Copper in solution			Copper adsorbed ($\mu g\ g^{-1}$)
	Ionic ($\mu g\ ml^{-1}$)	Total ($\mu g\ ml^{-1}$)	% complexed	
Humic acid	0.015	0.095	84	1990
	0.020	0.100	79	2240
	0.014	0.090	84	2491
	0.012	0.075	83	2742
	0.009	0.100	91	2990
	0.025	0.105	76	3240
Fulvic acid	0.006	0.265	97	1973
	0.012	0.385	97	2462
	0.013	0.440	97	2956
	-	0.460	-	3204

Figure 3.9.

The effect of complexing of copper in soil solution on adsorption isotherms



the gradients of the adsorption isotherms (table 3.14.). The figures indicate that the concentration of copper in natural soil solutions will be influenced most by organic materials and soil oxides, and the influence of some clay minerals may be negligible.

3.5.1.5. The influence of the background concentration of major cations

In view of the high degree of selectivity shown by soil materials for copper, extensive conventional ion exchange would appear unlikely to occur at the solution copper concentration range under study, even if the concentration of cations, such as calcium, in the soil solution was considerably lowered.

In order to investigate this, two experiments were carried out. Adsorption of copper by soil materials in the presence of 0.05M $CaCl_2$ was compared with adsorption in a medium of deionised distilled water and the effect on adsorption was examined of different calcium concentrations over the range likely to be found in soils (tables 3.15., 3.16.).

In the absence of $CaCl_2$, the solution salt contents would have been well below those found in most soils. The adsorption isotherms obtained (figure 3.10.) differed in slope from those obtained in the presence of $CaCl_2$ but the differences were small in relation to the large change in the ratio of competing ions to those of copper. The form that the differences took was, with clay and soil oxide, the expected increase in adsorption in the absence of calcium as the result of lack of competition for cation exchange sites. Humic acid, however, showed a corresponding decrease in adsorption. This is likely to be due to the solubilisation of some humic material and copper associated with it in the medium of lower ionic strength, a

Table 3.14.

Gradients of copper adsorption isotherms (soil materials)

Adsorbent	Gradient (ml g^{-1})	
Fulvic acid (extracted with NaOH)	117,200	(1)
Humic acid (extracted with NaOH)	97,900	(1)
Manganese oxide (sunthetic)	70,300	
Humic acid (extracted with NaOH)	51,600	(2)
Iron oxide (synthetic)	45,800	
Humic acid (extracted with $Na_4P_2O_7$)	38,300	(2)
Soil oxide (finely ground)	32,000	
Peat	19,000	
Soil oxide	14,200	
Fulvic acid (extracted with NaOH)	7,500	(2)
Fulvic acid (extracted with $Na_4P_2O_7$)	6,000	(2)
Montmorillonite	4,700	(3)
Illite	300	
Kaolinite	20	

(1) Based on ionic copper in solution

(2) Based on total copper in solution, including complexes

(3) Based on initial linear part of isotherm

Table 3.15.

Adsorption of copper by soil colloidal material in the presence and absence of 0.05M $CaCl_2$

Material	Medium	Initial Soln. Cu Conc. ($\mu g\ ml^{-1}$)	Equilibrium Soln. Cu Conc. ($\mu g\ ml^{-1}$)	Cu adsorbed by material ($\mu g\ g^{-1}$)
Humic acid	$CaCl_2$	10.40	0.036	1035.0
		5.18	0.022	516.8
		3.06	0.015	303.9
		2.00	0.012	198.7
		0.79	0.007	78.1
	Water	10.35	0.070	1025.0
		5.28	0.049	523.7
		3.13	0.039	308.3
		2.05	0.022	202.7
		0.76	0.014	74.4
Soil oxide	$CaCl_2$	3.06	0.020	302.5
		2.00	0.011	198.4
		0.99	0.006	98.1
		0.68	0.004	67.3
		0.47	0.003	46.6
	Water	3.13	0.015	313.7
		2.05	0.008	203.9
		0.97	0.004	96.4
		0.67	0.003	67.1
		0.44	0.002	43.7
Montmorillonite	$CaCl_2$	0.99	0.049	94.3
		0.47	0.009	46.2
		0.08	0.001	7.9
	Water	0.97	0.008	96.7
		0.44	0.003	43.7
		0.12	0.001	11.9

Table 3.16.

The effect of calcium concentration on adsorption of copper by soil materials

Material	Ca^{2+} Conc.	Initial Soln. Copper Conc. ($\mu g\ ml^{-1}$)	Equilibrium Soln. Copper Conc. ($\mu g\ ml^{-1}$)	Cu adsorbed by material ($\mu g\ g^{-1}$)
	0.05M	7.20	0.0362	68.5
	0.005M	"	0.0180	70.2
Montmorillonite	0.0005M	"	0.0136	70.5
	0.00005M	"	0.0135	70.5
	0.0	"	0.0147	70.6
	0.05M	16.40	0.0124	163.0
	0.005M	"	0.0086	162.9
Soil oxide	0.0005M	"	0.0090	163.3
	0.00005M	"	0.0077	163.4
	0.0	"	0.0064	163.9

Figure 3.10a.

Adsorption of copper by soil materials

in the presence and absence of 0.05M $CaCl_2$

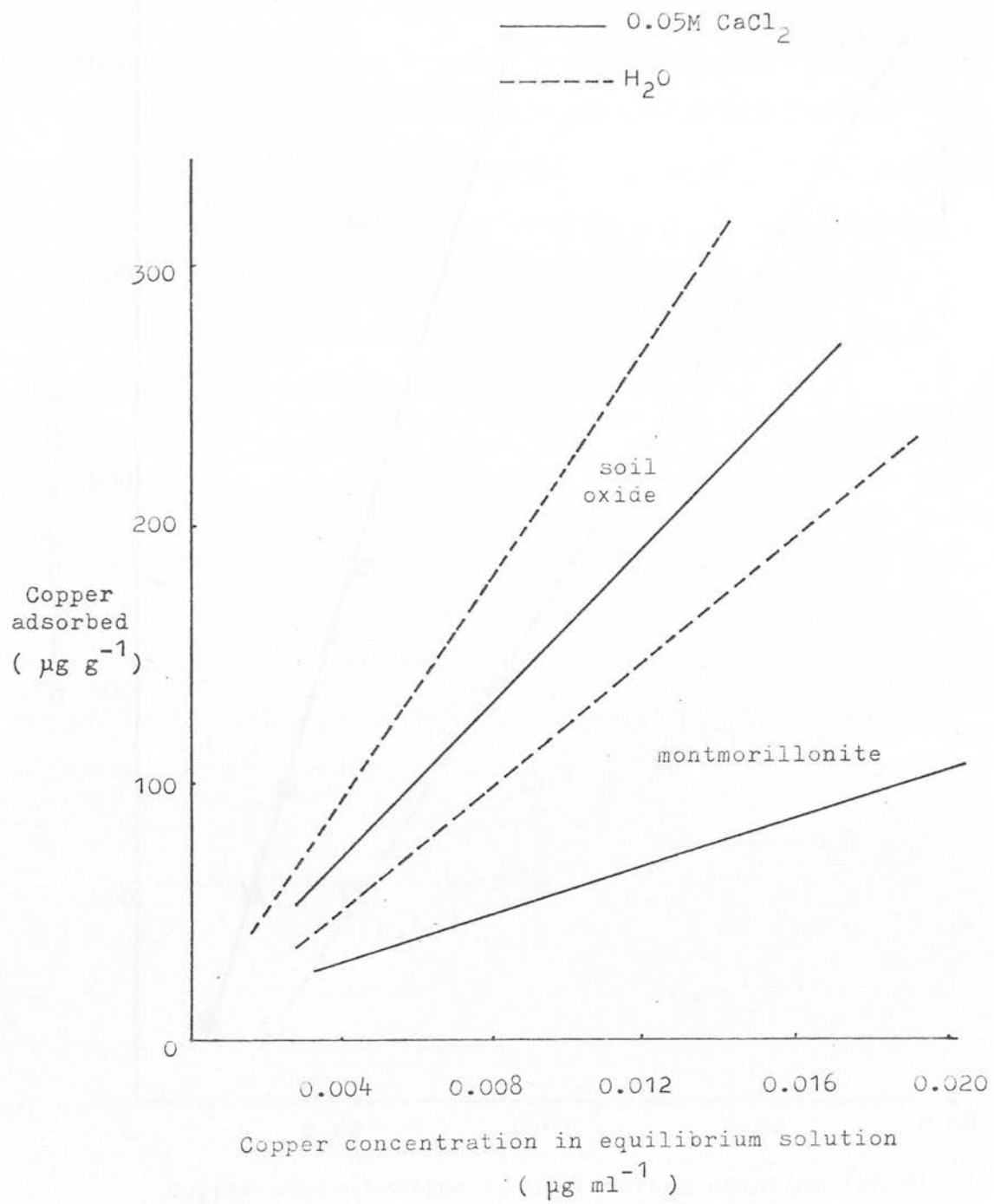
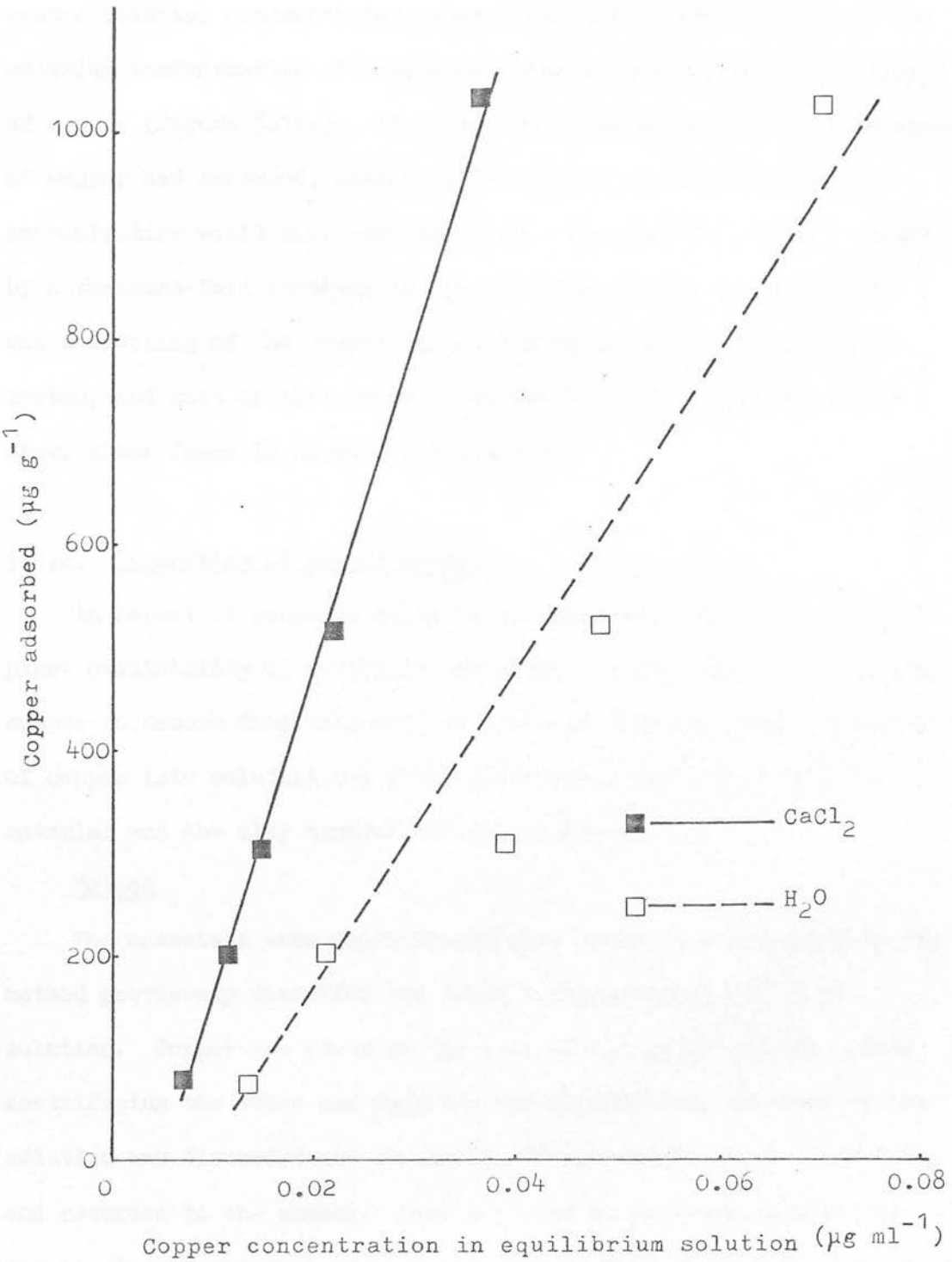


Figure 3.10b.

Adsorption of copper by humic acid in the presence and absence of 0.05M $CaCl_2$



state of affairs indicated by the higher optical density (at 400 nm) of this solution.

The second experiment confirmed that large changes in the background solution concentration caused relatively little change in the solution concentration of copper and thus in the degree of adsorption of copper (figure 3.11.). If significant conventional cation exchange of copper had occurred, considerable changes in solution copper concentration would have been observed. The greatest change, caused by a thousand-fold increase in the solution calcium concentration, was a doubling of the copper concentration in the montmorillonite system, and most of this change occurred at calcium concentrations above those found in normal soil solution.

3.5.2. Desorption of sorbed copper

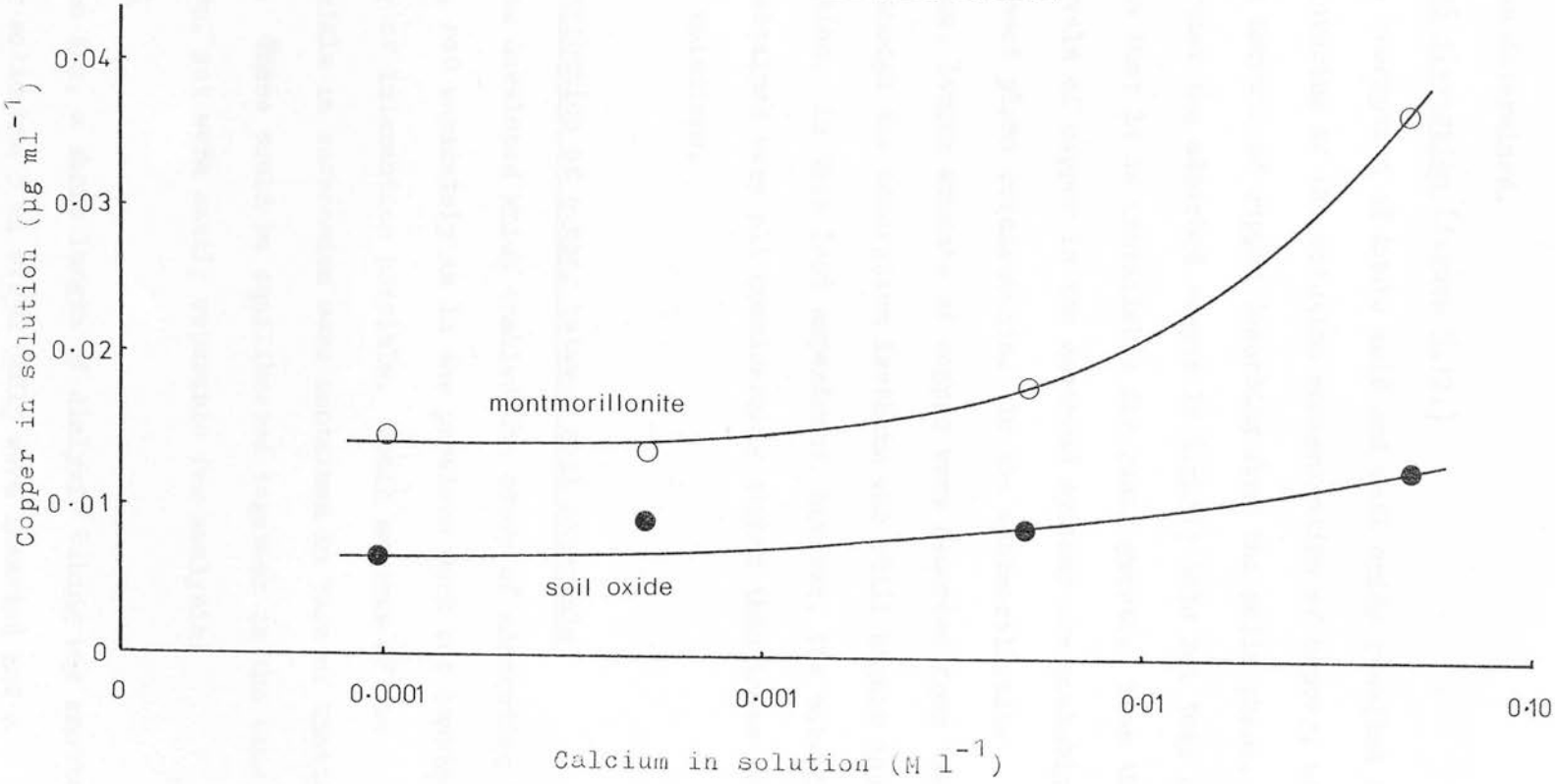
An aspect of sorption which is of great importance, as far as plant availability of copper is concerned, is the ability of adsorbed copper to desorb from soil colloid surfaces into solution. Desorption of copper into solution was studied for humic acid, soil oxide material and the clay mineral montmorillonite.

Method

The materials were equilibrated with copper and copper-64 by the method previously described but using 0.25g material and 25 ml solution. Copper was added at the rate of 0.3 $\mu g\ g^{-1}$ solid. After centrifuging the tubes and sampling the supernatant, the rest of the solution was discarded and the solid resuspended in 25 ml 0.05M $CaCl_2$ and returned to the shaker. Over a period of 24 hours, a total of six samples of supernatant were obtained in this way and the copper-64

Figure 3.11.

The effect of calcium concentration on the amount of copper in solution



activity in them determined.

Results and discussion (figure 3.12.)

Successive desorption of humic acid and soil oxide resulted in a substantial lowering of the solution concentration of copper, with only very small amounts of copper desorbing from the solid phase. This indicates that the adsorbed copper is tightly held but does not necessarily mean that it is unavailable for plant growth. Even the low solution levels of copper in the desorbed systems are probably high enough to meet plant requirements. In the montmorillonite desorption system, larger amounts of copper were desorbed from the solid phase, although the desorption isotherm was still higher than that for adsorption. In this last experiment, however, the solution concentrations obtained were all considerably higher than those found in natural soil solutions.

3.5.3. The distribution of copper between soil materials

A method was developed which enabled the study of adsorption by soil components, not separately as in the previous work but combined, making the study of interaction possible. Small amounts of the individual materials in suspension were contained in bags of knotted dialysis tubing. These could be equilibrated together in the same external solution, yet were easily separable for analysis.

3.5.3.1. Method

To form each bag, a short length of dialysis tubing was knotted at one end, 0.1g solid and 5 ml 0.05M $CaCl_2$ were inserted and a second knot was tied to seal the container. Bags containing humic acid, soil oxide and montmorillonite were placed in a 150 ml flask

Figure 3.12a.

Desorption of copper from humic acid

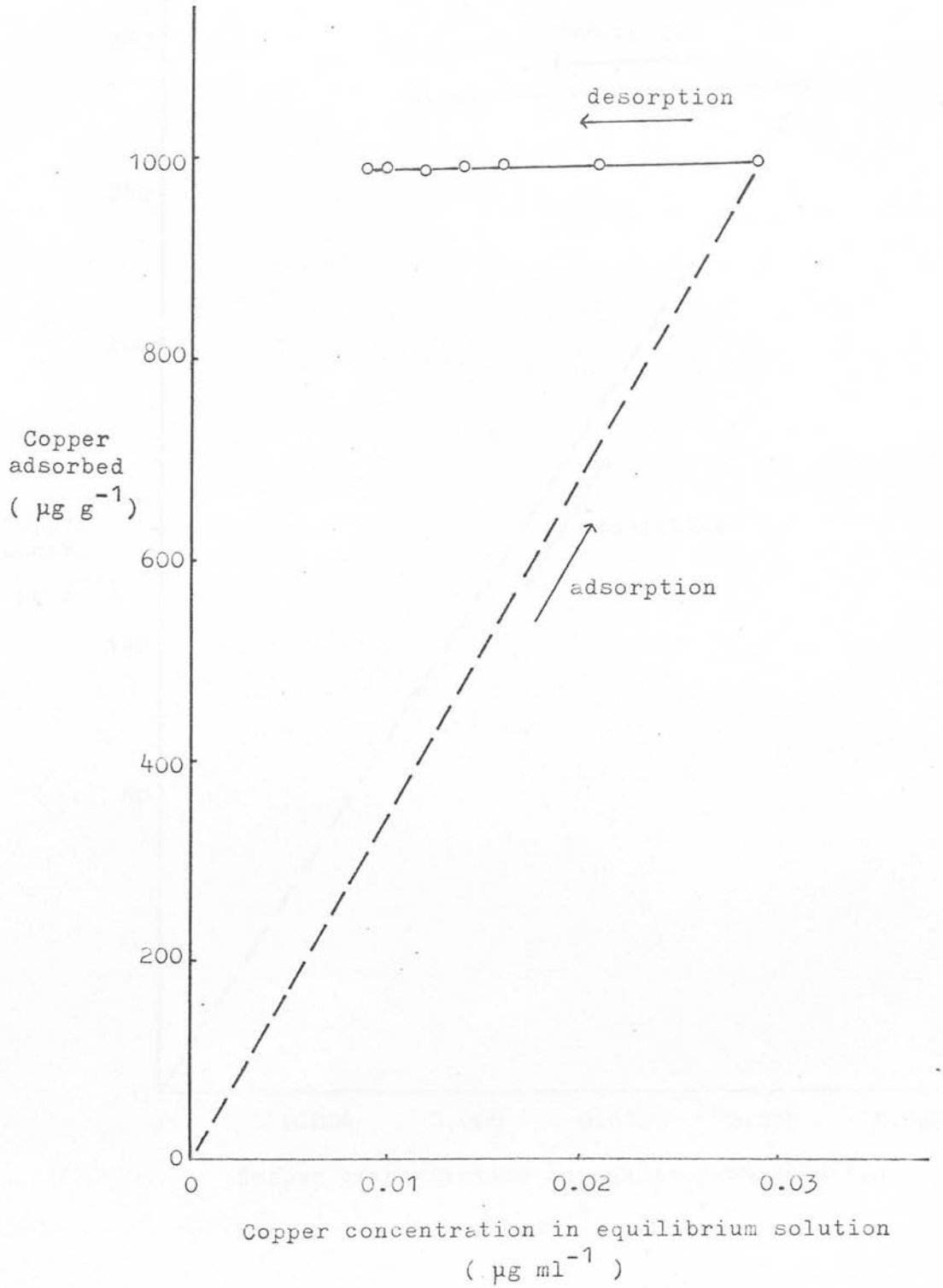


Figure 3.12b.

Desorption of copper from soil oxide

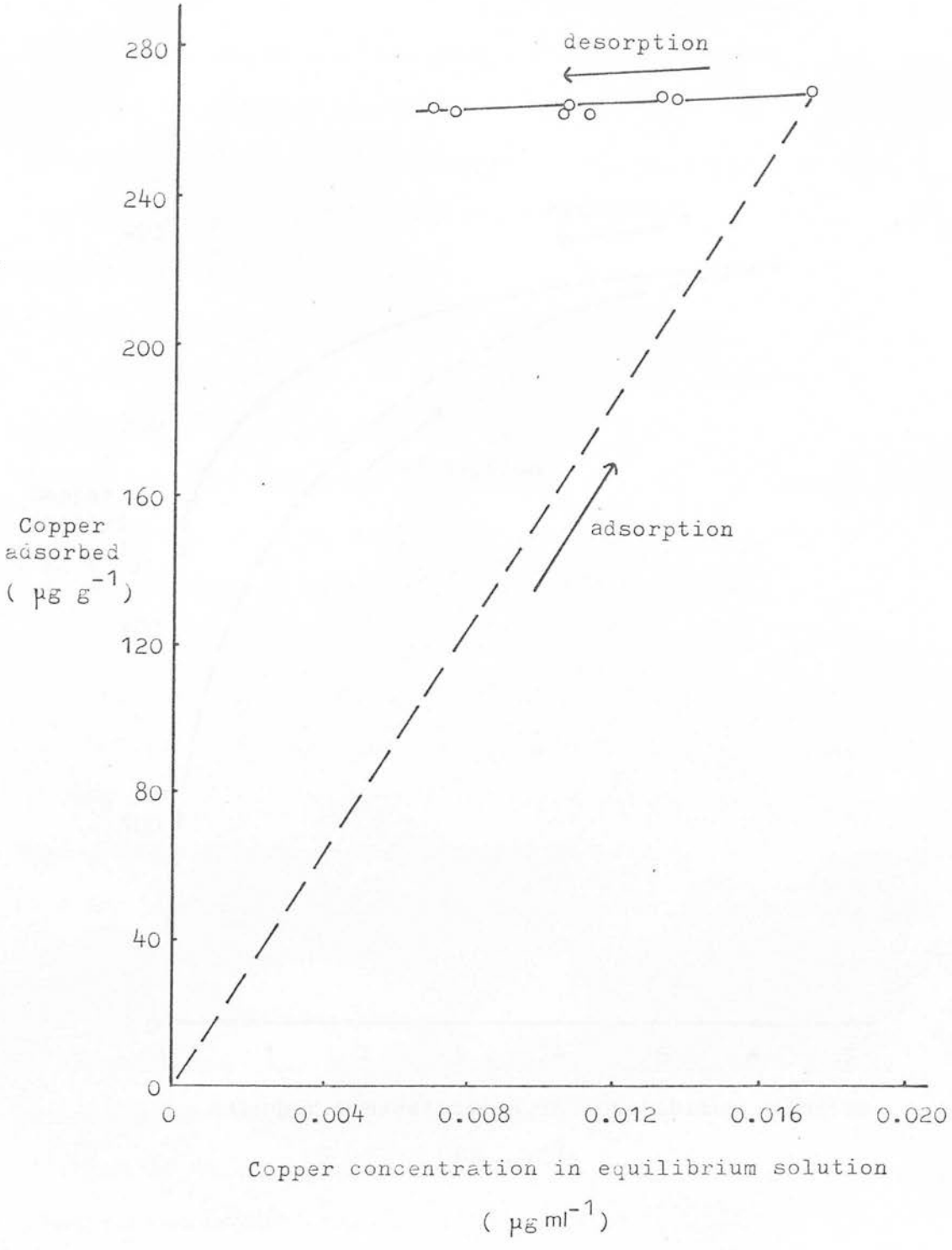
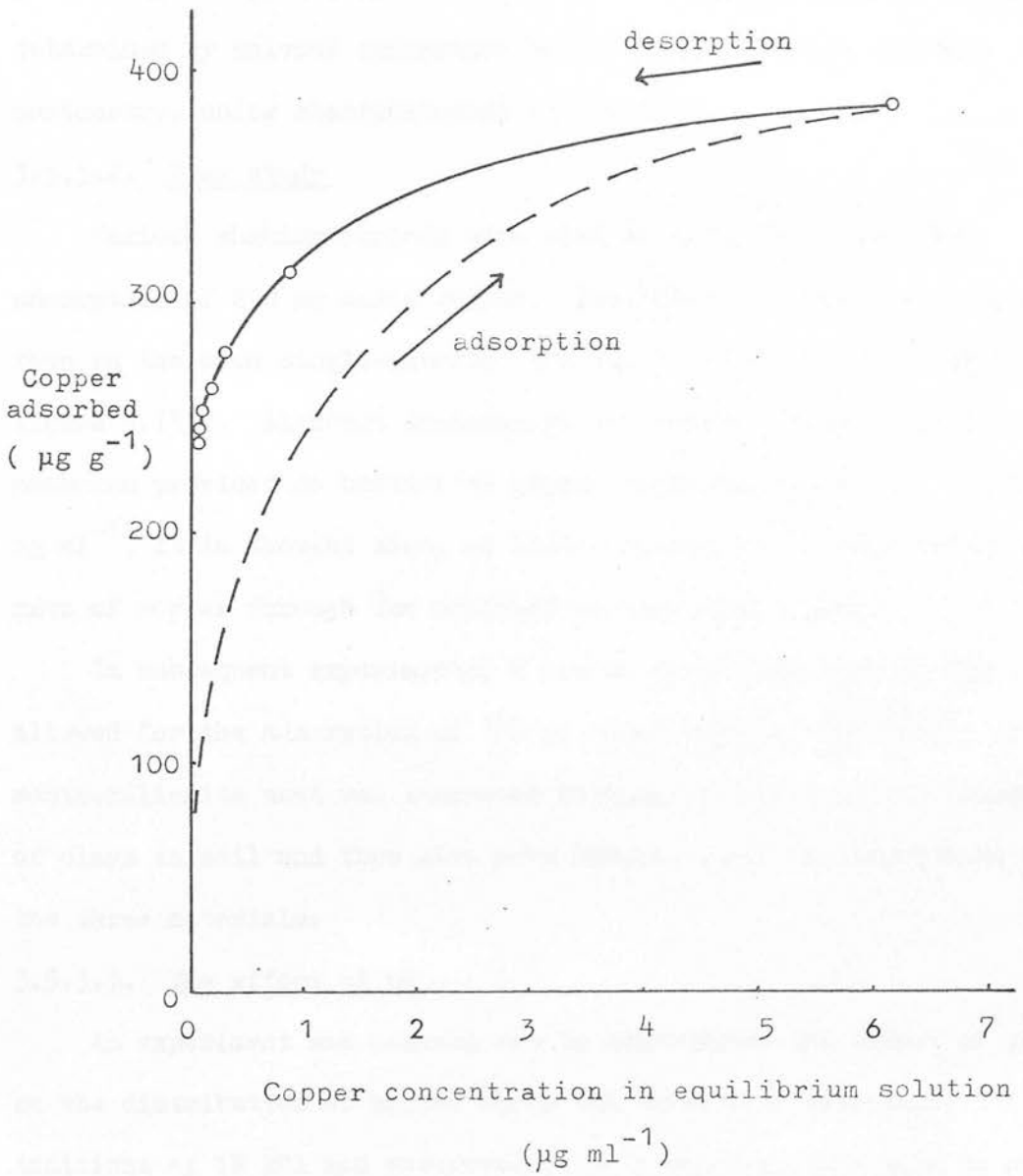


Figure 3.12c.

Desorption of copper from montmorillonite



containing 90 ml 0.05M $CaCl_2$, pH 6.0, with added copper. The flask was sealed and placed on an orbital shaker at 24°C. On the completion of the shaking period, each bag was removed and its contents washed into a platinum crucible. After evaporation to dryness, copper was determined by the HF digestion method used in soil fractionation (appendix 1), with the addition of prior dry ashing, at 450°C, of the humic acid. Copper concentrations in the external solutions were determined by solvent extraction and atomic absorption spectrophotometry, using electrothermal atomisation.

3.5.3.2. Time study

Various shaking periods were used in order to follow the adsorption of 200 µg added copper. Equilibration took much longer than in the open single-material systems previously used (table 3.17., figure 3.13.). Although preliminary experiments showed that the membrane provided no barrier to copper concentration of 0.1 - 1.0 µg ml^{-1}, it is thought that, at lower concentration, the diffusion rate of copper through the membrane is very much slower.

In subsequent experiments, a period of 21 days shaking was allowed for the adsorption of 100 µg added copper. The weight of montmorillonite used was increased to 0.5g to represent the abundance of clays in soil and thus give more natural relative proportions of the three materials.

3.5.3.3. The effect of pH

An experiment was carried out to investigate the effect of pH on the distribution of copper among the three soil materials. Additions of 1N HCl and saturated $Ca(OH)_2$ solution were used to obtain a range of pH values (table 3.18., figure 3.14.).

Table 3.17.

Time study of the adsorption of copper by soil materials enclosed in dialysis tubing

Time since copper addition (days)	Soln. copper conc. ($\mu g\ ml^{-1}$)	Copper adsorbed by solids ($\mu g\ g^{-1}$)		
		Humic acid	Soil oxide	Montmorillonite
1	0.638	756	547	147
2	0.338	961	602	158
4	0.094	1080	599	146
7	0.048	1120	626	135
11	0.040	1260	543	122
17	0.030	1270	564	106

Figure 3.13.

Time study of the adsorption of copper by soil materials enclosed in dialysis tubing

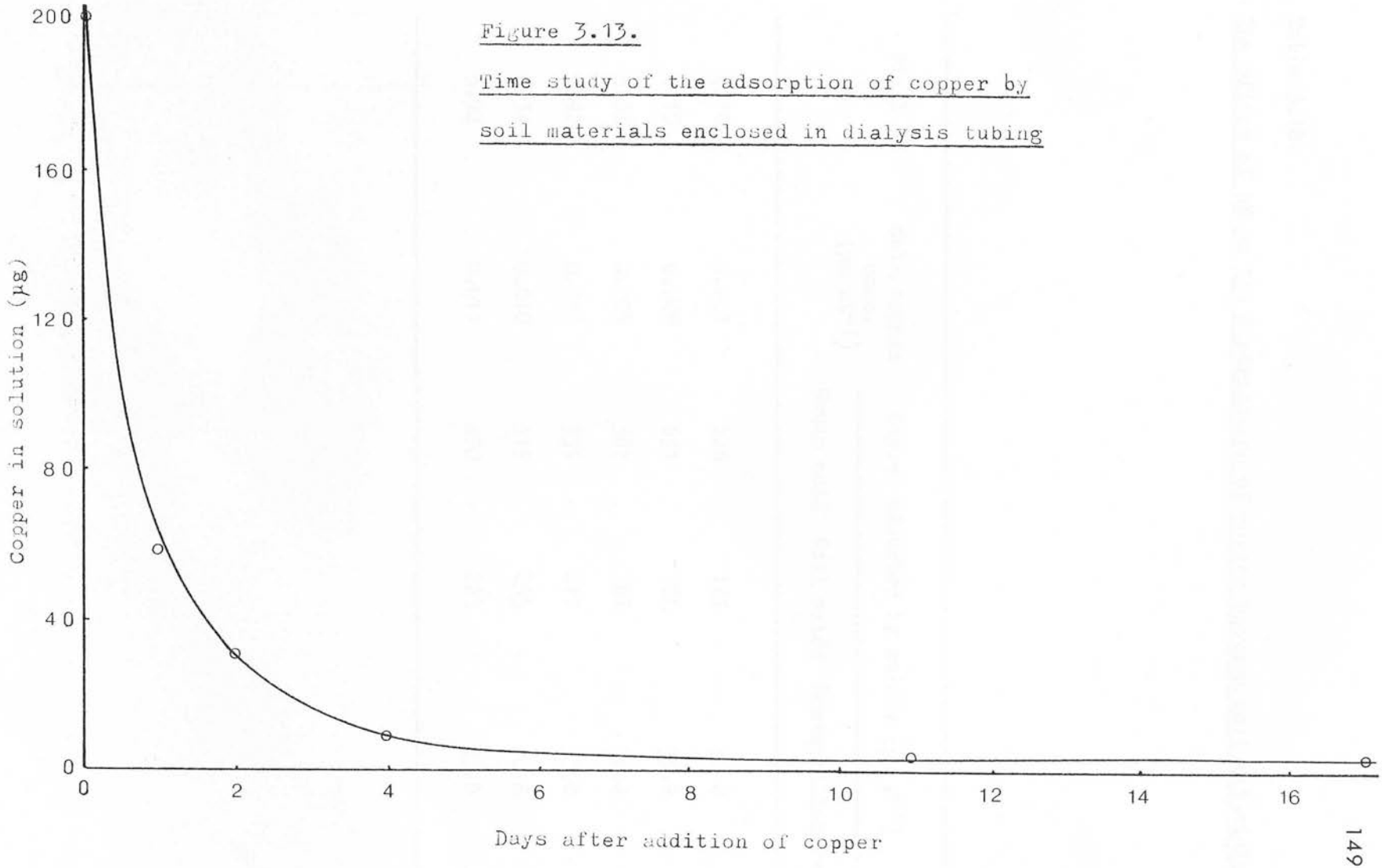


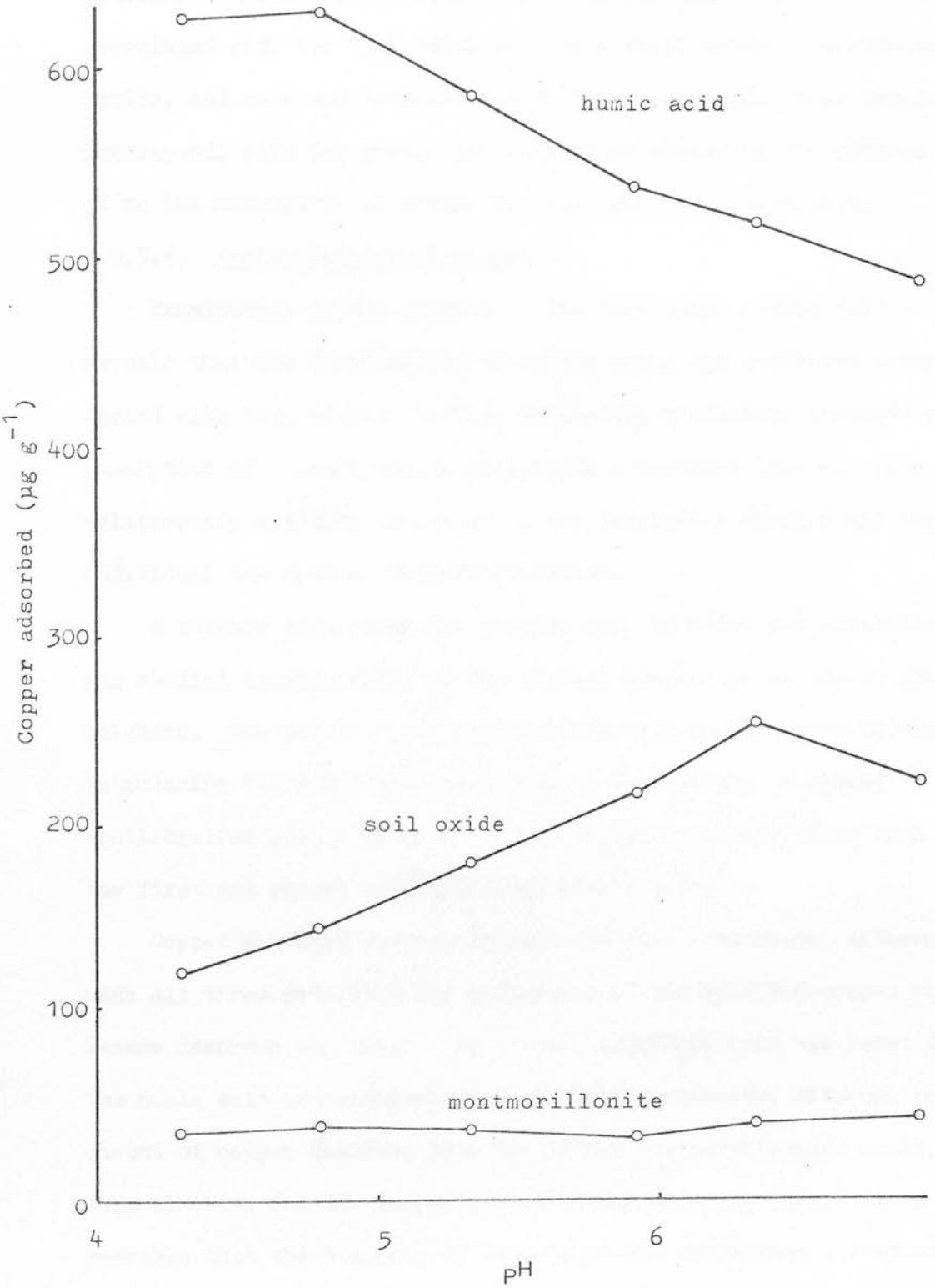
Table 3.18.

The effect of pH on the distribution of copper between soil materials

Final pH	Soln. copper conc. ($\mu g\ ml^{-1}$)	Copper adsorbed by solids ($\mu g\ g^{-1}$)		
		Humic acid	Soil oxide	Montmorillonite
4.30	0.011	626	121	36.0
4.79	0.008	631	128	39.2
5.33	0.009	587	181	36.4
5.92	0.010	539	217	33.0
6.34	0.010	519	255	40.8
6.92	0.011	490	223	43.8

Figure 3.14.

The effect of pH on the distribution of copper between soil materials



The solution copper concentration remained almost constant over the pH range but the distribution of copper between the different materials varied considerably. At lower pH values, less copper was associated with the soil oxide and, to a small extent, the montmorillonite, and more was associated with the humic acid. This trend corresponds with the prediction made after observing the effects of pH on the adsorption of copper by individual soil materials.

3.5.3.4. Redistribution of copper

Examination of the results of the time study (table 3.17.) reveals that the distribution of copper among the different materials varied with time (figure 3.15.), indicating a relative strength of adsorption of : humic acid > soil oxide > montmorillonite. This relationship was also indicated by the desorption studies and the individual adsorption isotherm gradients.

A further experiment was carried out, in which redistribution was studied independently of the initial adsorption of copper from solution. One material was equilibrated with added copper before introducing the other two components to the system. A second equilibration period followed. Samples were analysed after both the first and second equilibrations (table 3.19.).

Copper was able to move between the soil components, although with all three materials the proportion of the adsorbed copper which became desorbed was small. As before, redistribution was least from the humic acid and greatest from the montmorillonite, although the amount of copper desorbed from the latter was surprisingly small, even allowing for the larger amount of the material used. It is possible that the transfer of copper, at the prevailing low solution

Figure 3.15. Redistribution of copper with time

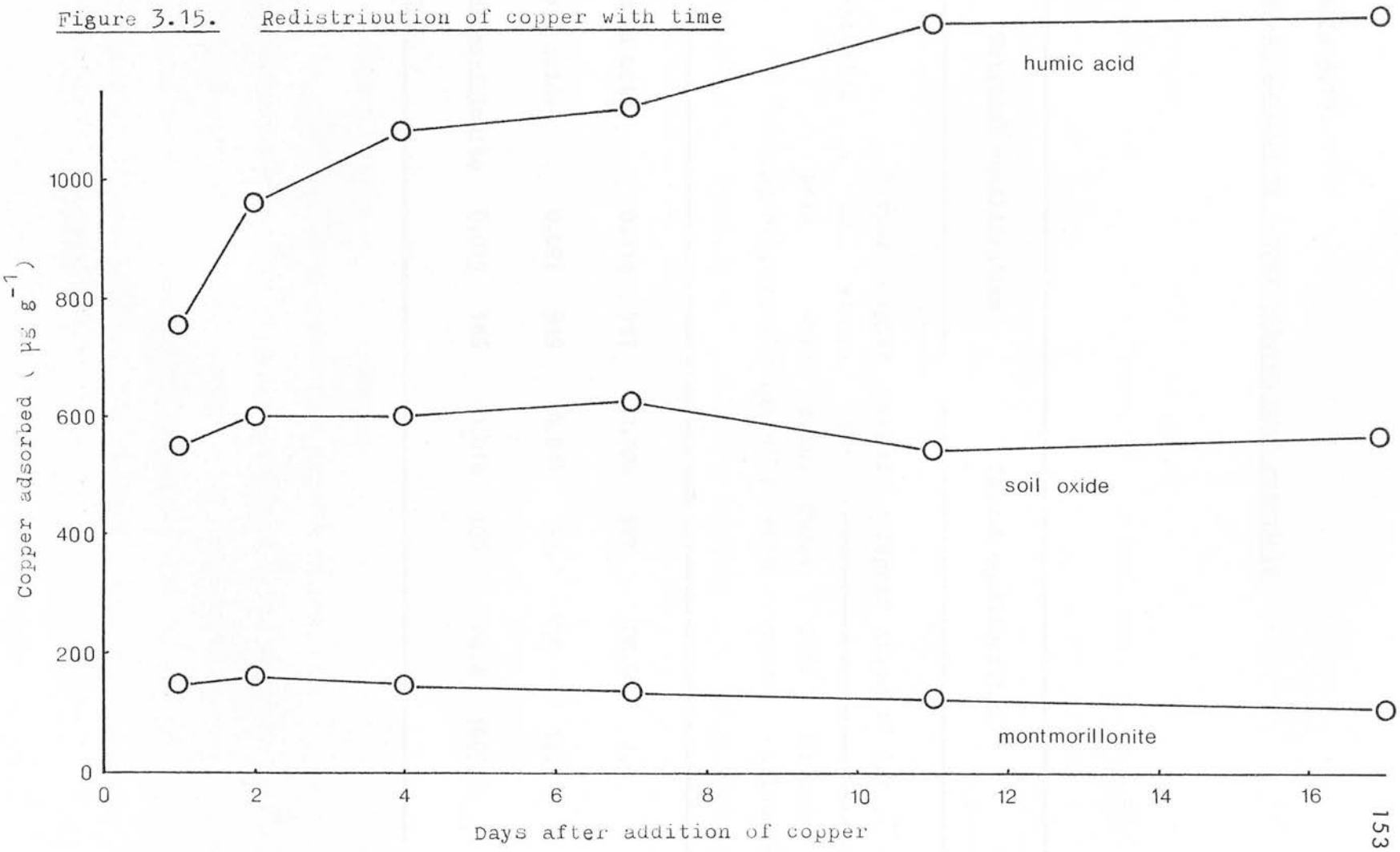


Table 3.19.

Redistribution of copper between soil materials

Original equilibration			Second equilibration			
Material	Copper in soln. ($\mu g\ ml^{-1}$)	Copper adsor-bed ($\mu g\ g^{-1}$)	Copper in soln. ($\mu g\ ml^{-1}$)	Copper adsorbed ($\mu g\ g^{-1}$)		
				Humic acid	Soil oxide	Montmor-illonite
Humic acid	0.014	987	0.006	987	28.9	4.3
Soil oxide	0.021	948	0.017	92.0	870	11.7
Montmorillonite	0.019	186	0.018	106	64.4	160

copper concentration, was slowed even further by the need for it to pass through two thicknesses of the dialysis membrane.

3.5.4. Isotopically exchangeable copper

The amount of copper released from any soil component in desorption or dialysis studies is only a small fraction of that ultimately capable of being mobilised. This is because, even at the high solution : solid ratio used, the reverse component of the reaction, Cu (solid) $\rightleftharpoons$ Cu (solution), predominates. Only if some solution copper were removed from the system would more solid-phase copper be mobilised.

The amount of copper which is isotopically exchangeable is a measure of the whole of the most mobile fraction of soil copper (that which readily equilibrates with solution phase copper). Samples of soil components were equilibrated with different levels of copper and the amounts of isotopically exchangeable copper present in the systems were subsequently determined. For comparison purposes, another estimate of the more labile soil copper (that extractable with EDTA) was also made.

Method

The method previously described for the determination of isotopically exchangeable copper was followed, with slight modifications. 0.4g soil component was used instead of 4g soil and up to 40 µg copper was added before the initial equilibration. Also, after sampling the final equilibrium solution, the remainder was discarded and the solid was extracted with 20 ml EDTA solution. The mixture was centrifuged and the activity and copper content of the supernatant were determined.

The amount of added copper adsorbed by the soil was calculated from the copper content of the $CaCl_2$ sample. Isotopically exchangeable copper was calculated as before and both this and the amount of copper extracted with EDTA were expressed as a percentage of the adsorbed copper. From the activity in the EDTA extract, the recovery of copper-64 was determined, with a small correction for the amount removed in the $CaCl_2$.

Results and discussion (table 3.20.)

Adsorption by montmorillonite and soil oxide fitted in well with previous results. However, adsorption by humic acid was low. The material used was obtained from a mineral soil, whereas that used in earlier experiments was extracted from peat. The darker colour of the solution phase after equilibration suggested a higher concentration of soluble organic matter, which, by increasing the amount of copper in the solution, would result in lower calculated values for adsorption.

At the level of copper addition common to all the components (100 $\mu g\ g^{-1}$), the total amounts of isotopically exchangeable copper appear similar with all three minerals, although the corresponding solution concentrations differ. However, of the copper added, the proportion which is isotopically exchangeable decreases in the order : montmorillonite > soil oxide > humic acid, reflecting the strength of adsorption by the minerals. EDTA, however, fails to reflect the variation between the materials, recovering a similar proportion of the added 100 μg Cu g^{-1} from all three.

In all cases, an increase in the amount of adsorbed copper results in an increased amount of labile copper, as measured both by isotopic exchange and extraction with EDTA. With humic acid, the

Table 3.20.

Isotopic exchange of copper adsorbed by soil components

Soil component	Cu added ($\mu g\ g^{-1}$)	Cu in solution ($\mu g\ ml^{-1}$)	Cu-IE (adsorbed)					Percentage of total copper-64 in $CaCl_2$ and EDTA extracts
			Cu adsorbed ($\mu g\ g^{-1}$)	($\mu g\ g^{-1}$)	% of adsorbed	EDTA-Cu (adsorbed) ($\mu g\ g^{-1}$)	EDTA-Cu (adsorbed) % of adsorbed	
Montmorillonite	0	0.0047	-	9.35	-	5.04	-	55.0
	50	0.0091	49.09	56.35	95.7	49.99	91.5	59.0
	100	0.0154	98.46	78.80	70.5	77.58	73.7	61.2
Soil oxide	0	0.0029	-	12.07	-	13.07	-	86.4
	100	0.0079	99.2	71.11	59.5	82.0	69.5	90.4
	200	0.0135	198.7	129.5	59.1	184.5	86.3	90.5
Humic acid	0	0.0160	-	30.1	-	30.9	-	87.9
	100	0.0259	97.6	70.1	41.0	101.3	72.3	91.5
	200	0.0430	195.9	138.1	55.1	202.5	87.7	91.6

percentage of adsorbed copper remaining labile increases as the amount of copper adsorbed increases, probably because copper is held less firmly at higher levels of site saturation. With montmorillonite, however, there is a corresponding decrease in the proportion of the copper determined as labile. Possibly, at the higher levels of copper addition, the element is diffusing further into the mineral lattice. With soil oxide, the percentage of adsorbed copper extractable with EDTA increases with the amount adsorbed but the percentage which is isotopically exchangeable does not vary, showing that the two methods of assessing availability can lead to different conclusions.

With humic acid and soil oxide, EDTA extracts a greater percentage of adsorbed copper than is isotopically exchangeable, the difference being greater with the former material. Thus a large part of EDTA-extractable copper is not involved in immediate replenishment of copper in the solution phase and is not strictly readily available.

Although the amounts of copper extracted with EDTA tend to be greater than that which is isotopically exchangeable, not all of the copper-64 associated with the solid phase was recovered by EDTA, suggesting that some copper which is isotopically exchangeable is not extractable with EDTA. With humic acid and soil oxide only about 10% of the copper-64 is unextractable but a larger amount (over 40%) of the activity added to montmorillonite was not recovered. A simple explanation for the latter figure, indicating that EDTA functions poorly as an extractant of copper from clay, is suggested by the observation of Martin and Reeve (1957) that large amounts of clay in soil samples reduce the efficiency of EDTA. This would also explain why the amount of copper extractable with EDTA from montmorillonite

did not exceed the amount which was isotopically exchangeable.

An alternative explanation for the poor recovery from montmorillonite is suggested by previous results (figure 3.6.), which indicate that the montmorillonite system would not have been at equilibrium when the copper-64 was added. Adsorption, rather than isotopic exchange of copper, is likely to have occurred, resulting in the Cu-IE values obtained being a considerable over-estimate of the readily available copper. A very much smaller degree of adsorption, and possibly some experimental error, could also explain the incomplete recovery of copper-64 from the other soil materials.

3.5.5. Conclusions

All the materials examined selectively adsorb copper but adsorption by oxides and organic materials is much greater than that shown by clay minerals. Thus, even in soils of high clay content, it is likely that control of the copper concentration in solution will be by the other constituents. The linear nature of the adsorption isotherms indicates that, within the solution copper concentration range found in normal soils, the relative importance of the various materials will not vary. Within this copper concentration range, the equilibrium between adsorbed and solution phase copper is not greatly influenced by the concentration of cations, such as Ca^{2+}, in the soil solution. Increase in acidity beyond pH 5 significantly increased solution copper concentrations in oxide and clay systems but organic materials were not similarly affected. It is likely that, in natural soils, copper released from clays and oxides by a fall in pH would be adsorbed by organic matter, rather

than causing an increase in solution copper levels.

Although adsorption reactions are obviously important in influencing solution levels of copper, the humic and fulvic acid studies showed that total solution concentrations of copper can be greatly enhanced, above the equilibrium levels for ionic copper, by the presence of soluble organic complexes. Hodgson et al (1965, 1966) suggested that up to 99% of the copper in soil solutions may exist in complexed forms. If this proves to be the general case, levels of ionic copper in soil solutions will be considerably lower than the total copper levels, emphasising the need for studying copper adsorption at low equilibrium solution copper levels. It could also mean that, since only ionic copper was involved in the clay and oxide systems described above, such materials are less important in adsorbing copper in soils, compared with the organic materials, than the results at first suggest. The actual distribution of copper between the various solid phase constituents will, of course, be unaffected by the presence of soluble complexes.

The ease with which adsorbed copper can desorb into solution will be of major importance in determining the long-term availability of copper. Studies indicated that, although adsorbed copper is tightly held, sufficient desorption probably occurs to maintain solution copper levels and thus the supply to plants. Although montmorillonite released most copper, soil oxide and humic acid will be more important in long-term maintenance of the level in solution.

Results from a system containing several soil materials supported the idea of redistribution of copper as a result of a decrease in pH and confirmed the relative strengths with which the

various materials adsorb copper.

Comparison of the amount of copper extractable by EDTA with that which is isotopically exchangeable, reveals that the former technique is less sensitive to variations in the strength with which copper is adsorbed by different materials. It overestimates the amount of copper involved in maintaining solution levels, the size of the error depending on the materials involved.

3.6. Adsorption and isotopic exchange of copper by soils

The study of adsorption by individual soil materials has suggested their relative importance in the overall adsorption and availability of copper in soils. In order to determine if the net properties of soils in these respects correspond with those expected from their composition, some of the techniques previously used were applied to eleven intact soils. The influence of pH on the size of the soluble and isotopically exchangeable fractions of soil copper was also investigated.

3.6.1. Method

The method used for determining the size of the isotopically exchangeable pool of copper associated with soil components was applied to soils. 4g air-dry soil which had been passed through a 2 mm sieve was used instead of 0.4g soil component and the number of different levels of copper included in the adsorption equilibration was increased. The emphasis was still, however, on obtaining equilibrium copper concentrations similar to those found in normal soil solution.

With two of the soils and some of the copper treatments the amounts of soluble and isotopically exchangeable copper were determined at a range of pH values, obtained by added 0.1N HCl or saturated $Ca(OH)_2$ to the tubes before the initial equilibration.

The characteristics of the eleven soils used are given in table 3.3.

3.6.2. Adsorption

Adsorption isotherms for the soils, like those obtained for individual soil components, were linear (figure 3.16). The gradients were determined by regression analysis (table 3.22.). The individual correlation coefficients were all significant when $P < 0.001$.

The gradients of the adsorption isotherms are all low, the values being of the same order of magnitude as those obtained with pure clays. This is because only a fraction of the total soil weight is active in adsorption. If the gradients are determined, for example, relative to the weight of organic matter in each sample, the gradient values obtained are similar to those determined for the more active soil components.

The adsorption gradients fall into two groups, i.e. 5, 31, 45 and 49 and the remainder, the latter being associated with the steeper gradients. The division does not appear to be associated with any soil characteristics, apart from the mean manganese content being 2.5 times larger for the steeper group of gradients.

No significant correlation was found between the gradients of the adsoprtion isotherms and any single soil characteristic. However, soil pH and organic matter, iron and clay contents were intercorrelated, with several of the correlations significant at the 5% level.

There was no significant multiple correlation between the gradients of the adsorption isotherms and any combination of soil pH, organic matter, Mn, Fe and clay contents. Some multiple correlations which were significant at the 10% level were obtained. These included equations in which increase in adsorption isotherm gradient was related to increase in soil Mn content or pH and decrease in $CaCl_2$-

Table 3.21.

Adsorption and isotopic exchange of copper by soils

Soil	Cu added ($\mu g\ g^{-1}$)	Cu in solution ($\mu g\ ml^{-1}$)	Cu adsorbed ($\mu g\ g^{-1}$)	Cu-IE(adsorbed)		EDTA Cu(adsorbed)	
				($\mu g\ g^{-1}$) *	% of Cu adsorbed	($\mu g\ g^{-1}$) *	% of Cu adsorbed
1	0	0.0009	-	0.60	-	1.24	-
	1	0.0014	0.99	1.16	56.6	2.17	93.9
	2	0.0017	1.98	1.56	48.5	2.99	88.8
	5	0.0034	4.97	3.46	57.5	5.45	85.1
	8	0.0059	7.94	6.01	68.1	8.04	85.6
	20	0.0130	18.97	11.99	57.3	18.09	84.8
2	0	0.0013	-	0.71	-	2.18	-
	1	0.0024	0.98	1.47	77.6	2.94	77.6
	2	0.0024	1.98	1.61	45.5	3.86	84.8
	5	0.0033	4.97	2.44	34.8	6.48	86.5
	8	0.0057	7.94	4.72	50.5	8.90	84.6
	20	0.0110	19.89	12.96	61.6	19.30	84.7
3	0	0.0014	-	0.98	-	1.89	-
	1	0.0018	0.98	1.47	50.0	2.74	86.7
	2	0.0025	1.98	2.10	56.6	3.56	84.3
	5	0.0043	4.96	3.82	57.3	6.22	87.3
	8	0.0070	7.93	6.92	74.9	8.66	85.4
	20	0.0125	19.88	11.98	55.1	18.62	84.2
4	0	0.0035	-	1.69	-	2.68	-
	1	0.0037	0.96	1.98	30.2	3.50	85.4
	2	0.0047	1.95	2.66	49.7	4.44	90.3
	5	0.0065	4.94	4.20	50.8	7.01	87.7
	8	0.0082	7.92	5.89	53.0	9.63	87.8
	20	0.0146	19.85	13.86	61.3	19.74	85.9
5	0	0.0031	-	1.02	-	1.98	-
	1	0.0041	0.96	1.60	60.4	2.80	85.4
	2	0.0048	1.95	2.04	52.3	3.67	86.7
	5	0.0079	4.92	3.98	60.2	6.18	85.4
	8	0.0112	7.89	6.17	65.3	8.61	84.0
	20	0.0224	19.78	14.49	68.1	18.27	82.4
6	0	0.0018	-	0.38	-	0.35	-
	1	0.0037	0.96	1.30	95.5	1.31	99.7
	2	0.0047	1.95	2.00	83.1	2.17	93.2
	5	0.0067	4.93	3.33	59.8	4.84	91.1
	8	0.0088	7.91	4.56	52.8	7.18	86.3
	20	0.0141	19.86	11.49	55.9	17.41	85.9

* Includes native soil copper

Table 3.21. (continued)

Soil	Cu added ($\mu g\ g^{-1}$)	Cu in solution ($\mu g\ ml^{-1}$)	Cu adsorbed ($\mu g\ g^{-1}$)	Cu-IE(adsorbed)		EDTA Cu(adsorbed)	
				($\mu g\ g^{-1}$) *	% of Cu adsorbed	($\mu g\ g^{-1}$) *	% of Cu adsorbed
31	0	0.0039	-	1.40	-	1.77	-
	1	0.0049	0.95	2.01	64.2	2.53	80.0
	2	0.0051	1.95	2.36	49.2	3.43	85.1
	5	0.0083	4.92	4.25	57.9	5.96	85.2
	8	0.0120	7.88	6.50	64.7	8.16	81.1
	20	0.0224	19.78	13.25	59.9	18.62	85.2
33	0	0.0025	-	0.81	-	2.11	-
	1	0.0034	0.97	1.16	36.1	2.93	84.5
	2	0.0040	1.96	1.81	51.0	3.73	82.7
	5	0.0059	4.94	2.90	42.3	6.43	87.4
	8	0.0065	7.94	3.69	36.3	8.91	85.6
	20	0.0128	19.8	9.62	44.3	18.84	84.2
35	0	0.0021	-	0.94	-	1.61	-
	1	0.0037	0.96	1.85	94.8	2.46	88.5
	2	0.0047	1.95	2.35	72.3	3.37	90.3
	5	0.0064	4.94	3.53	52.4	5.83	85.4
	8	0.0097	7.90	5.70	60.3	8.13	82.5
	20	0.0175	19.83	11.87	55.1	18.79	86.6
45	0	0.0028	-	0.85	-	1.75	-
	1	0.0046	0.95	1.55	73.7	2.46	74.7
	2	0.0052	1.95	1.81	49.2	3.06	67.2
	5	0.0074	4.93	3.17	47.1	5.86	83.4
	8	0.0113	7.89	5.26	55.9	8.37	83.9
	20	0.0237	19.76	14.18	67.5	18.57	85.1
49	0	0.0023	-	0.45	-	0.99	-
	1	0.0035	0.97	1.01	57.7	1.80	83.5
	2	0.0052	1.95	1.73	65.6	2.73	89.2
	5	0.0094	4.91	3.77	67.6	5.33	88.4
	8	0.0127	7.87	5.63	65.8	7.97	88.7
	20	0.0265	19.74	14.27	70.0	18.39	88.1

Figure 3.16.

Examples of soil adsorption isotherms

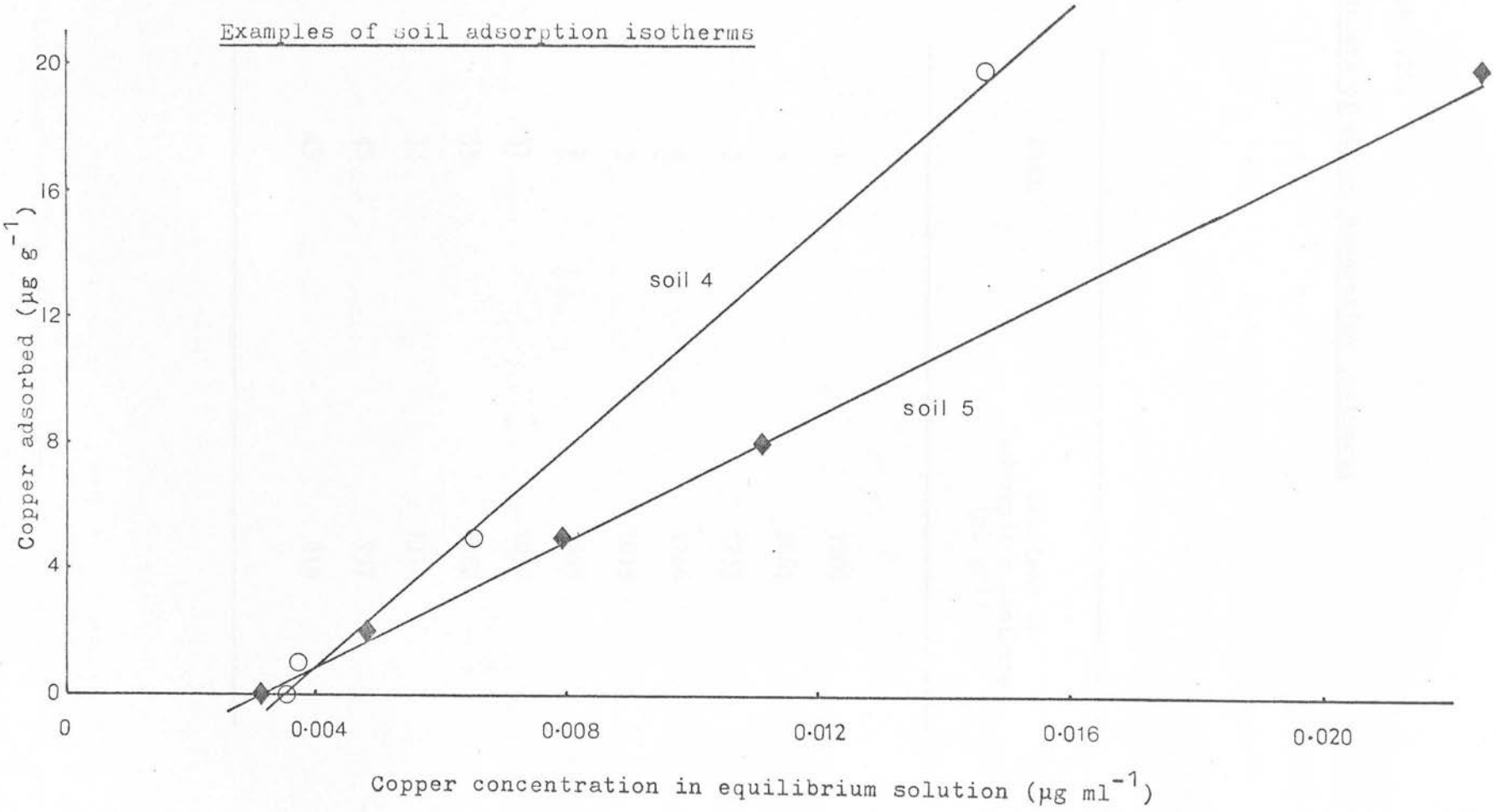


Table 3.22.

Gradients of copper adsorption isotherms

Soil	Gradient of adsorption isotherm ($ml\ g^{-1}$)
1	1609
2	2061
3	1735
4	1766
5	1019
6	1655
31	1050
33	1982
35	1312
45	957
49	818

extractable copper.

The absence of significant correlations may have been due to several factors. The size of the soil collection was small, making statistically significant relationships less likely. In addition, the range of values of different soil characteristics, such as organic matter, Fe and Mn contents, was limited. This is probably because all the soils came from the same area of southern Scotland, so that the amount of variation in parent material and conditions of formation is relatively small. In contrast, McLaren and Crawford (1973b), who obtained a significant multiple correlation between adsorption of copper and soil organic matter and manganese contents, used a much wider range of soils, from 16 series and with considerably more variation in soil characteristics. The above-named authors were more likely to obtain significant correlations for an additional reason. This is that their measure of adsorption was the maximum adsorption capacity of a soil, which is more likely to be related to the composition of the soil than is an index of adsorption obtained with only a small percentage of the possible adsorption sites occupied.

3.6.3. Isotopic exchange

The percentage of adsorbed copper remaining isotopically exchangeable or extractable by EDTA (table 3.21.) seemed to be relatively independent of the amount of copper adsorbed, in spite of a twenty-fold variation in site coverage. The results from the experiments with individual soil components suggested otherwise, although the extent of saturation of sites on actively adsorbing constituents is unlikely to have differed greatly between the two

experiments. However, the earlier studies indicated that, in some cases, an increase in the amount of copper adsorbed by a material is associated with a decrease, rather than an increase, in the proportion which is labile (table 3.20.). Thus, variations associated with individual materials may cancel each other out, resulting in variation in the figures for complete soils being relatively small.

The lack of variation between copper treatments allows the values to be averaged for each soil (table 3.23.). In all cases, EDTA extracted considerably more copper than was isotopically exchangeable, as it did with humic acid and soil oxide material alone. Thus it appears that EDTA generally over-estimates the amount of readily-available copper. There is little variation between the different soils, suggesting a fundamental similarity in the adsorption of copper, but neither set of values is significantly correlated with any single soil characteristic.

With all soils, approximately 10% of the added copper-64 was not recovered by extraction with EDTA. There was no evidence of the large retention observed previously with montmorillonite, which could have caused a large error in the results.

3.6.4. The effect of variations in pH

Within the range 4.2 - 6.4, the pH of the system had no effect on the copper concentration in the equilibrium solution (figure 3.17.). This is in agreement with the prediction made from the results of studies of individual materials that, at low solution copper levels, variation in pH over the range found in most agricultural soils has little effect on copper availability. In more acid conditions, the

Table 3.23.

Mean availability of adsorbed copper,

as determined by isotopic exchange and EDTA extraction

Soil	Cu-IE, as % of adsorbed Cu	CuEDTA, as % of adsorbed Cu
1	57.6	87.6
2	54.0	83.6
3	58.8	85.6
4	49.0	87.4
5	61.3	84.8
6	69.4	91.2
31	59.2	83.3
33	42.0	84.9
35	67.0	86.7
45	58.7	78.9
49	65.7	87.6

Table 3.24

The effect of pH on soluble and isotopically exchangeable soil copper

Soil	Added copper ($\mu g\ g^{-1}$)	pH	Copper in solution ($\mu g\ ml^{-1}$)	Copper adsorbed ($\mu g\ g^{-1}$)	Isotopically exchangeable copper (adsorbed)	
					($\mu g\ g^{-1}$)	%
33	0	3.27	0.0055	-	1.35	-
		4.23	0.0025		1.33	
		5.21	0.0030		1.60	
		5.63	0.0026		1.08	
		6.17	0.0039		1.43	
		6.99	0.0086		1.79	
45	0	3.12	0.0061	-	1.42	-
		4.21	0.0015		0.63	
		5.42	0.0026		0.97	
		6.01	0.0025		0.85	
		6.39	0.0035		1.13	
		7.27	0.0085		2.16	
45	5	3.17	0.0265	4.73	4.05	55.2
		4.25	0.0074	4.93	3.59	60.0
		5.38	0.0061	4.94	3.47	50.7
		6.02	0.0074	4.93	3.47	50.7
		6.29	0.0077	4.93	4.27	63.7
		6.80	0.0132	4.86	6.92	125.7
		7.24	0.0207	4.79	8.21	126.3

Figure 3.17.

The effect of pH on $CaCl_2$-extractable soil copper

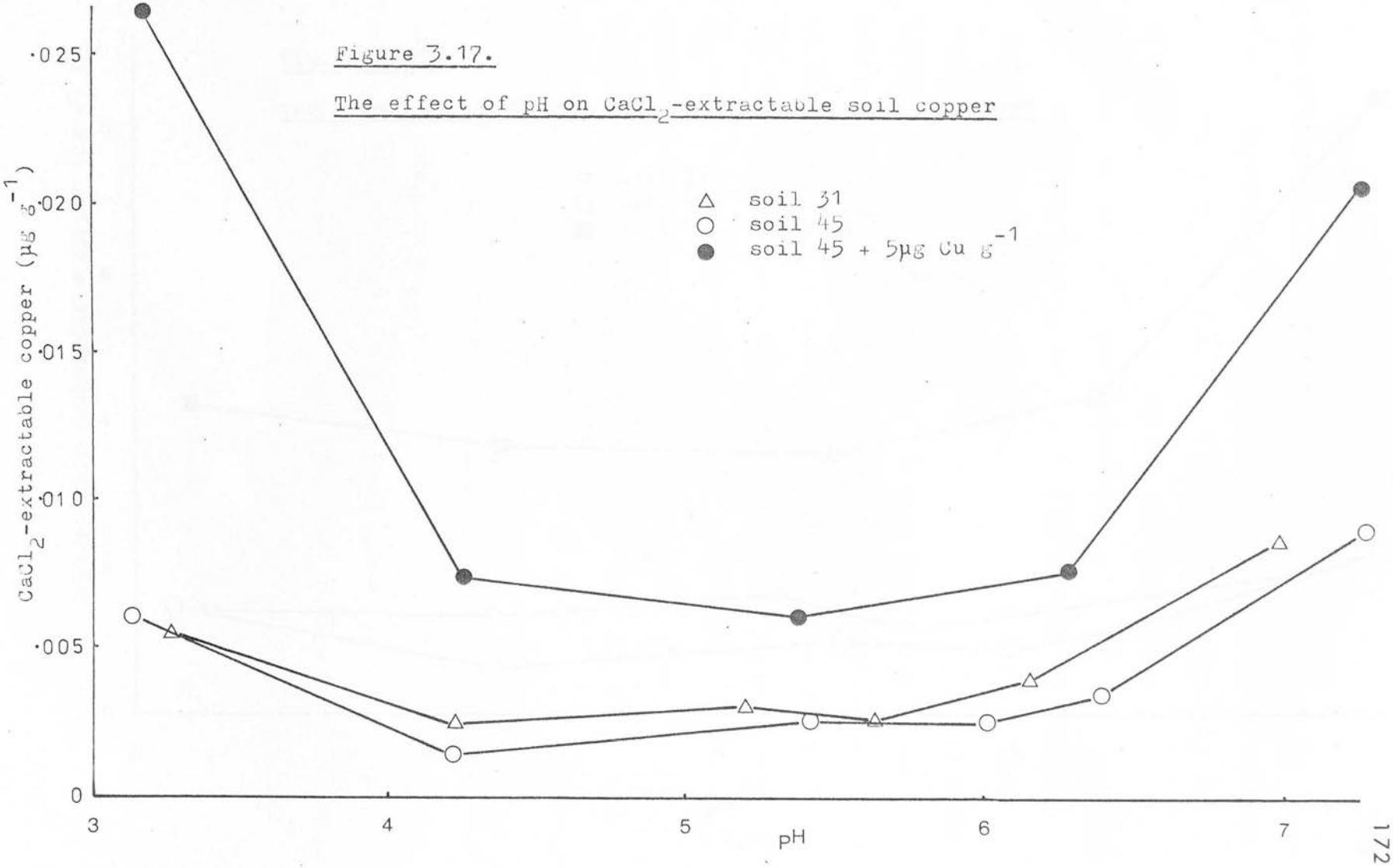
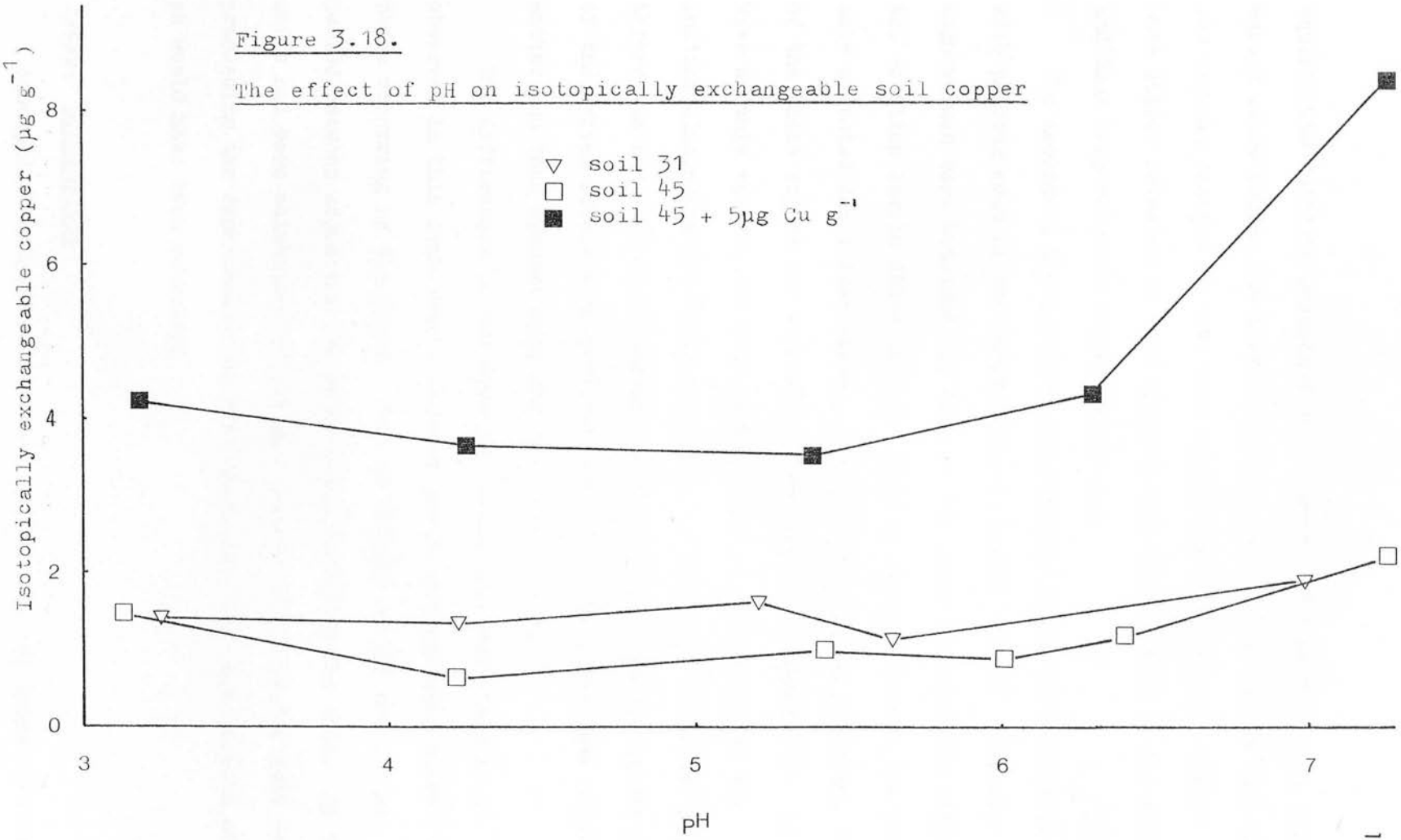


Figure 3.18.

The effect of pH on isotopically exchangeable soil copper



equilibrium solution contained more copper. The increase was slight, except where copper was added to soil no. 45. It is likely that the low organic content of this soil limits its capability to adsorb both copper released by soil minerals and copper added to the system and thus keep solution copper levels low.

The amount of isotopically exchangeable copper varied little with pH over most of the range studied (figure 3.18,). However, high values were obtained for both isotopically exchangeable copper and solution levels above pH 6.5. This is unusual because low pH only affected the latter value. Also, at the highest pH value, 126% of the added copper was apparently isotopically exchangeable. In view of this result, the high values could be the result of the initial adsorption not being completed, in the tubes at higher pH, before the addition of copper-64. Alternatively, increasing the pH of the system before equilibration may have solubilised some organic matter and thus brought more copper into solution.

The differences in the measured values with variation in pH observed in this experiment, although small, are probably higher than those occurring in the field. This is because of the short-time period between adjusting the soil pH and assessing the soil. If the soils had been maintained at the appropriate pH values for some weeks preceeding the experiment, the more important long-term effects of pH would have been revealed.

3.6.5. Conclusions

Adsorption isotherms for soils, as with most of those obtained with the individual components, were linear. The gradients of the

isotherms reflected the small proportion of the materials in the soils which adsorb copper strongly but were not significantly correlated with any single soil characteristic. Multiple regression analysis did not reveal any strongly significant trends, probably because of the small number of soils used.

Neither isotopically exchangeable nor EDTA-extractable copper varied as a proportion of the amount of copper adsorbed, suggesting the involvement of only one type of site. However, it is possible that variations associated with individual soil components cancelled themselves out. Both methods of assessment indicated that there was little variation in the availability of the copper added to the different soils.

Over the pH range found in normal soils there is little variation in the levels of soluble soil copper and thus variation in soil soil solution copper levels with pH is unlikely. The size of the isotopically exchangeable pool of soil copper appears not to be influenced by soil pH.

3.7. The effect of copper additions to soils on plant copper uptake

The distribution of native and applied copper between the solid and solution phases of a soil will affect its availability to plants. Although the types of reaction processes involved when copper is added to the soil can be studied in the laboratory, as demonstrated above, their effects on copper availability to plants are difficult to predict. This is partly because it is necessary in laboratory experiments to use high solution to soil ratios. As a result, equilibration of copper is likely to take place more rapidly than under the conditions existing in the field.

Assessment of the amount of available copper in a soil by chemical extraction also involves a high solution to soil ratio, which gives the extractant far greater contact with the soil than the roots of a plant have under normal conditions. Thus, the estimate obtained is likely to be high. Growing plants in soil implies a system with a natural soil to solution ratio. The presence of plants makes the system more complicated but does allow direct assessment of the amount of copper that plants actually take up from the soil.

Although a single rate of copper application is recommended for all copper-deficient mineral soils, this amount can be increased up to five times for organic soils (E.S.C.A., 1975). Thus it is unlikely that the lower rate will be the optimum addition for all mineral soils and the increasing cost of applying copper makes it desirable to avoid the need to repeat additions without applying unnecessarily large amounts. This will only be possible when more is known about the response of crops on different soils to a variety of levels of added

copper. It should not, however, be necessary to investigate all soils in this manner. Investigation of responses using a relatively small number of well-characterised soils could enable predictions of crop response to be made for other soils, from values for selected characteristics known to affect the response to copper additions.

Six soils, samples of which had been used in previous studies, were treated with different rates of copper and used in a pot trial designed to investigate the effect of the treatments on the copper content of red clover.

3.7.1. Method

The experiment was laid out in a randomised block design with six soils and five different copper treatments (table 3.25.). Each soil/copper treatment combination was replicated three times (3 blocks). The soils used (table 3.3., appendix 3 : nos. 1 - 6) were passed through a 6.25 mm sieve to remove stones. Treatment involved adding the appropriate amount of copper, in 500 ml water, to 20 kg portions of soil in a small concrete mixer and mixing for ten minutes. Each batch filled three 9 inch pots (6.25 kg each) and provided a separate sample for analysis. One pot of each set was placed at random on each of three benches in an unheated greenhouse.

A late flowering red clover (Trifolium pratense L. var. Merkur) was sown at the rate of 0.1g seed per pot. Distilled water was supplied both to the surface and via individual plastic saucers, with the latter predominating after the initial weeks. One application of a nutrient solution containing N, P and K (appendix 5) was made after twelve days.

Early slow growth was followed by a thickening out of the plants

Table 3.25.

Pot trial 1 : Rates of application of copper

Treatment	kg $CuSO_4.5H_2O$ ha^{-1}	mg $CuSO_4.5H_2O$ pot^{-1}	µg Cu g^{-1} soil
1	0	0	0
2	20	49.1	2.29
3	49	123	5.75
4	98	246	11.52
5	196	491	23.64

in all soils except no. 6. The foliage was harvested in late August, after 9 weeks of growth. At the same time, soil samples were taken for analysis. The yield of dry matter from each pot was recorded and the herbage copper content determined. The amounts of copper extracted from the various soils with EDTA were determined both before and after plant growth.

3.7.2. Results and discussion

In the report on this and subsequent pot trials significant differences, relative to the controls unless otherwise stated, are indicated thus :

* : $P < 0.05$

** : $P < 0.01$

*** : $P < 0.001$

3.7.2.1. EDTA-extractable soil copper (table 3.26.)

Where no copper was added, levels of EDTA-extractable copper apparently increased slightly during the period of the trial. This is probably due to experimental error, although there is a possibility, indicated by the results of Bromfield (1958) and Nielsen (1976b), that root exudates might have caused the increase. In 19 out of the 24 treatments involving soil to which copper had been added, there was a decrease in the amount of EDTA-extractable copper during the trial. Copper in the harvested foliage amounted to, at most, 0.2 $\mu g\ g^{-1}$ of the decrease and it is unlikely that all of the remainder was in the plant roots. Possibly redistribution of the added copper within the soil took place, resulting in some of it no longer being extractable with EDTA, as was observed to occur in the incubation

Table 3.26.

Pot trial 1 : Values determined

Soil	Treatment	EDTA-Cu($\mu g\ g^{-1}$)		Mean vegetation yield ($gDM\ pot^{-1}$)	Mean vegetation yield ($\mu g\ g^{-1}DM$)	Significance of Cu content compared to controls
		Before Trial	After Trial			
1	1	1.5	1.6	6.5	10.5	
	2	3.1	3.1	8.9	11.0	
	3	5.3	5.5	7.9	11.8	
	4	10.1	9.9	6.6	12.3	*
	5	20.7	21.3	8.1	12.4	
2	1	2.3	2.3	14.6	7.0	
	2	4.6	3.9	16.4	7.4	
	3	6.4	6.4	14.8	8.1	
	4	11.9	11.3	14.6	8.3	*
	5	20.2	18.3	14.0	8.5	**
3	1	1.8	1.9	14.7	8.7	
	2	4.4	3.7	14.2	9.5	*
	3	7.3	5.7	15.2	10.2	**
	4	13.0	9.9	13.3	10.9	***
	5	23.3	18.8	11.9	11.4	***
4	1	2.7	3.0	16.2	6.0	
	2	4.2	4.3	12.7	6.0	
	3	6.0	6.7	18.3	7.5	*
	4	11.2	10.9	16.0	7.7	**
	5	24.3	19.0	15.1	7.3	*
5	1	2.3	2.7	13.3	5.9	
	2	4.0	4.2	15.9	7.5	*
	3	6.9	6.8	15.8	7.7	**
	4	12.7	11.2	13.7	7.6	**
	5	20.1	19.5	15.1	8.8	***
6	1	0.40	0.45	3.2	4.2	
	2	2.9	2.1	3.4	7.2	***
	3	5.9	4.8	4.5	8.5	***
	4	10.1	9.2	5.5	8.5	***
	5	20.8	18.3	4.5	9.8	***

studies.

3.7.2.2. Plant yields

There was considerable variation between the three replicates of each treatment but it is clear from the means of the data (table 3.26.) that plant yield did not vary with the amount of copper added. This was expected, since, except in severe cases of copper deficiency, fertility factors other than copper will control yield.

The mean plant yields for individual soils were found to be significantly correlated ($r = 0.953^{**}$) with the EDTA-extractable copper values for the untreated soils. However, on those soils which gave the lowest yields, the yields were not improved by copper additions, so the correlation must be regarded as spurious.

3.7.2.3. Copper concentrations in clover plants

Mean values for each treatment are given in figure 3.26. An analysis of variance, appropriate to the randomised block, 2 factor factorial experiment, was carried out on the individual values (table 3.27.). This showed that :

(1) The blocks were not significantly different.

(2) Both the soils and the copper treatments gave significantly different vegetation copper concentrations.

(3) There was a slight interaction between soils and copper treatments, indicating that the individual soils responded differently to the copper treatments in terms of vegetation copper concentration.

The significance of differences between individual treatment means was determined by reference to the 't' distribution (table 3.26.). The minimum soil addition of copper required to significantly increase the concentration of copper in clover varied considerably among the

Table 3.27.

Pot trial 1 : Analysis of variance for plant copper concentrations

Source of variation	Degrees of freedom	Sum of squares	Mean of squares	Variance ratio	Significance
Blocks	2	3.052	1.526	2.968	**
Soils	5	251.03	50.21	97.68	**
Rate of copper	4	83.95	20.99	40.84	**
Interaction	20	23.99	1.20	2.33	*
Error	58	29.835	0.514		

six soils, from less than 20 kg ha^{-1} (soils 5 and 6) to 98 kg ha^{-1} (soils 1 and 2). It is likely that there would have been a significant response to the recommended rate of copper application of 25 kg $CuSO_4 . 5H_2O$ ha^{-1} on only one of the soils, no. 6. This is also the only one of the soils used with an EDTA-extractable copper value within the range normally accepted as indicating deficiency.

From the point of view of cattle nutrition, however, the copper status of the foliage grown on most of the soils was evidently poor. Without copper additions only one soil produced clover with a copper concentration over the dietary minimum for cattle (10 $\mu g\ g^{-1}$ dry matter - A.R.C., 1965). When the soils were supplemented with 50 kg $CuSo_4 . 5H_2O$ ha^{-1}, this level was reached on a second soil, but increasing the supplement fourfold added only one more soil, the "deficient" one, to the list. Five of the soils produced, without supplementation, clover containing sufficient copper for sheep (5 $\mu g\ g^{-1}$ dry matter - A.R.C., 1965). However, the copper concentration of mixed pasture would have been considerably lower than that determined in clover and most of the other plants in the sward would be less responsive than clover to increases in soil copper (Mitchell et al, 1957). Nevertheless, even a small increase in herbage copper concentration is of value, since it reduces the dependance of stock on other forms of copper supplement.

3.7.2.4. The effect of soil factors on copper concentrations in clover

The mean increase in clover copper concentration as a result of supplementing each soil with copper was calculated and correlations were sought between these values and textural and other characteristics of the soils. The only significant correlation obtained was with

native EDTA-extractable soil copper ($r = 0.863^{*}$). This correlation was negative and indicates that additions of copper were more effective on soils of low copper status. Correlation between the increases in plant copper and the soil adsorption isotherm gradients was surprisingly poor ($r = 0.224$).

Attempts were also made to find correlations between the copper concentrations of clover grown in the untreated soils and soil properties. Significant positive correlations were found with soil organic matter ($r = 0.967^{**}$), clay ($r = 0.962^{**}$), iron ($r = 0.898^{*}$) and silt ($r = 0.870^{*}$). Significant negative correlations were found with soil sand content ($r = 0.942^{**}$) and pH ($r = 0.897^{*}$). However, there are extensive correlations between these properties, probably as a result of using only a small number of soils in the trial. This makes it necessary to differentiate between those characters which are directly linked and those which are not, before any relationship can be accepted.

Unfortunately, the small number of soils also limits the value of multiple correlations, since an increase in the number of parameters included reduces the number of degrees of freedom. In this case, if four parameters are used, there is 1 degree of freedom.

Two multiple correlations were obtained which were significant at the 5% level. If a = copper content of control plants, b = $CaCl_2$ extractable soil copper, c = soil adsorption isotherm gradient, d = soil Mn and e = isotopically exchangeable soil copper, then :

$$a = 28.39 - 426.5b - 0.011c + 0.027d \quad (r = 0.99^{*})$$

and $$a = 736.9 - 0.45c - 316e \quad (r = 0.99^{*})$$

The negative function of the adsorption isotherm gradient in both

equations is logical but the negative values for available soil copper are unlikely, as is the increase in soil Mn content in the second equation. Again it is likely that false equations have resulted from the small sample size.

3.7.3. Conclusions

The growth of plants had some effect on the availability of soil copper as estimated by extraction with EDTA. Plant yield was not altered by the addition of copper to the soils. The relationship observed between yield and EDTA-extractable soil copper in this experiment is probably spurious.

Vegetation copper concentration was significantly modified by both soil type and the different rates of added copper. Statistical analysis also showed that the effect of copper additions on vegetation copper concentration varied from soil to soil. Thus, this trial has demonstrated that a single rate is not the optimum copper supplement for a range of mineral soils.

Only one soil was indicated, by extraction with EDTA, to have a copper content within the range normally accepted as "deficient". This soil was also the only one which would have benefitted from the recommended rate of copper application. Much larger additions of copper would be necessary to all of the soils to give herbage containing sufficient copper for cattle.

Little was learnt as to which soil properties control vegetation copper concentrations. This was probably because the trial involved only a small number of soils collected from a limited area. It is likely that a similar pot trial, using a larger number of soils

encompassing a wider range of properties, would yield more information on this subject.

3.8. The effect of adding chelated and non-chelated copper, cobalt and manganese to a soil on the uptake of those nutrients by plants

In the previous pot trial the form in which copper was added - $CuSO_4.5H_2O$ - was constant, as was the way in which the copper was mixed into the different soils. Copper is normally added to the soil in an inorganic form, usually as copper sulphate. In recent years, however, chelates such as CuEDTA have become available and claims have been made that these are more effective. The availability of different forms of copper added to soil requires investigation.

Copper applied to the ground surface penetrates only a short distance into the soil (Jones and Belling, 1967; Leite and Skogley, 1979) and may not be accessible to the roots of many plants. The availability of copper is known to be affected by variations in levels of macronutrients but the effects of micronutrients other than copper are relatively unknown. Cobalt and manganese are two elements which are often deficient in soils which are deficient in copper and thus might be applied simultaneously with copper.

A pot trial, involving a single soil and crops of barley, clover and ryegrass, was designed with the following objectives :

1) To compare the effect of additions of copper sulphate and different rates of copper EDTA to a deficient soil on uptake of copper by plants (barley, ryegrass and clover).

2) To examine the effect, on copper uptake by barley, of adding different forms and rates of manganese to the soil and supplying manganese as a foliar spray.

3) To examine the effect on copper uptake by ryegrass and

clover of additions of cobalt sulphate and cobalt EDTA to the soil.

(4) To compare the effects, on copper uptake by ryegrass and clover, of adding copper, as $CuSO_4$, to the soil in three different ways, i.e. (a) incorporated into the soil before sowing, (b) added to the soil surface immediately after sowing, and (c) added to the soil surface after plant establishment.

3.8.1. Method

The soil used came from a pasture site known to be deficient in copper (soil 6, table 3.3.). Treatment solutions containing copper, manganese and cobalt were made up in 200 ml distilled water. The sulphates of these elements were applied at the rates currently recommended by the East of Scotland College of Agriculture. The lower EDTA rates were based on the manufacturer's recommendations for soil treatment and the higher EDTA values were calculated to contain the same amount of the element as the sulphate treatments. The soil was treated in the same way as in the previous pot trial and the filled pots were placed on benches in an unheated greenhouse.

Three benches were used, one for each species. On each bench three replicate pots of each soil treatment to be used were placed at random. 30 seeds of barley (Hordeum vulgare L. var. Golden promise), perennial ryegrass (Lolium perenne L. var. S23) or red clover (Trifolium pratense L. var. S123) were spread evenly over the surface of each pot and covered with a little untreated soil. One treatment, which involved the addition of copper after sowing, was carried out by applying the copper in 100 ml water. 200 ml nutrient solution containing N, P and K was applied to each pot (appendix 5). The pots

Table 3.28.

Pot trial 2 : Rates of application of salts and chelates

A total of 20 different treatments were used. Each comprised either one or two of the additions detailed below.

Application	Compound	kg compound ha^{-1}	mg compound pot^{-1}	µg metal g^{-1} soil
Mixed into soil	$CuSO_4.5H_2O$	25	53.6	2.54
"	CuEDTA	2	4.3	0.08
"	"	64.8	139	2.54
"	$MnSO_4.4H_2O$	20	42.9	1.97
"	MnDTPA*/MnEDTA	6	12.9	0.34
"	" "	88.2	189.3	4.99
"	$CoSO_4.7H_2O$	6	12.9	0.5
	CoEDTA	1	2.1	0.04
"	"	12.5	26.8	0.5
Applied to soil surface	$CuSO_4.5H_2O$	25	53.6	-
Foliar spray	$MnSO_4.4H_2O$	5.6	12	-

* Diethylenetriaminepentaacetic acid

were watered with distilled water, via the soil surface until the plants were well established and then via individual plastic saucers. The foliar treatment of $MnSO_4$ was applied with a hand sprayer in two stages, after 27 and 52 days of growth.

The barley was harvested after 72 days. Three cuts were taken of the clover and ryegrass, after 88, 131 and 206 days, with further applications of nutrient solution being made after the first and second cuts. Plant yields were recorded after drying and the plant material analysed for copper, manganese and iron. In the case of the barley it was only possible to harvest and analyse the straw.

Samples of the treated soils used in the trial were analysed for EDTA- and $CaCl_2$-extractable copper. EDTA-extractable copper was also determined in soil samples collected at the end of the trial.

3.8.2. Results and discussion

Estimates of soil copper are from analysis of a single sample, which, in the case of the analysis carried out after the trial, was composed of bulk samples from individual pots.

After analysis of the vegetation from each pot, an analysis of variance appropriate to the randomised design was carried out on the individual values. The significance of differences between treatment means (tabulated) was determined by reference to the t-distribution.

3.8.2.1. Soil properties (table 3.29.).

Before the trial, more of the copper added as CuEDTA was extractable with EDTA (66.9% recovery) than that which was added as $CuSO_4$ (40.4% recovery). This suggests that there was less adsorption of the organic form. After the trial, however, the proportion of

Table 3.29.

Pot trial 2 : Levels of extractable soil copper

Crop	Treatment	Before pot trial		After pot trial
		$CaCl_2$ Cu ($\mu g\ g^{-1}$)	EDTA Cu ($\mu g\ g^{-1}$)	EDTA Cu ($\mu g\ g^{-1}$)
Barley (straw)	Control	<0.001	0.4	
	$CuSO_4$	0.003	1.5	
	$MnSO_4$	<0.001	0.5	
	$CuSO_4$ + $MnSO_4$	<0.001	1.3	
	$CuSO_4$ + MnEDTA(low)	0.003	1.7	
	$CuSO_4$ + MnEDTA(high)	>0.025	2.1	not determined
	$MnSO_4$(foliar spray)	0.001	0.4	
	$CuSO_4$ + $MnSO_4$(foliar spray)	0.003	1.5	
	CuEDTA(low) + $MnSO_4$(foliar spray)	<0.001	0.4	
	CuEDTA(high) + $MnSO_4$ " "	0.001	2.1	
	$CuSO_4$ + MnDTPA(low)	0.004	1.2	
	$CuSO_4$ + MnDTPA(high)	>0.025	1.6	
Clover	Control	< 0.001	0.4	0.4
	$CuSO_4$	0.003	1.5	2.3
	$CoSO_4$	0.002	0.4	0.4
	$CuSO_4$ + $CoSO_4$	0.003	1.6	2.4
	$CuSO_4$ + CoEDTA(low)	0.002	1.2	2.1
	$CuSO_4$ + CoEDTA(high)	0.004	1.4	2.3
	CuEDTA(low)	< 0.001	0.4	0.6
	CuEDTA(high)	0.001	2.1	2.2
	$CuSO_4$ applied to surface	< 0.001	0.4	0.7
	$CuSO_4$ applied to surface after first cut	< 0.001	0.4	0.5
Ryegrass	Control	< 0.001	0.4	0.5
	$CuSO_4$	0.003	1.5	2.5
	$CoSO_4$	0.002	0.4	0.6
	$CuSO_4$ + $CoSO_4$	0.003	1.6	2.6
	$CuSO_4$ + CoEDTA(low)	0.002	1.2	2.7
	$CuSO_4$ + CoEDTA(high)	0.004	1.4	2.4
	CuEDTA(low)	< 0.001	0.4	0.6
	CuEDTA(high)	0.001	2.1	2.5
	$CuSO_4$ applied to surface	< 0.001	0.4	0.6
	$CuSO_4$ applied to surface after first cut	< 0.001	0.4	0.6

both forms which was extractable had increased to a similar level (78.7% of the CuEDTA, 80.7% of the $CuSO_4$). Gilkes and Lim-Nunez (1979), using the same extractant, obtained the lower figures of 43% and 55% respectively. Both sets of values indicate that, after plant growth, chelated copper is no more available than the ionic form.

In experiments involving the dry storage and moist incubation of similar soils (section 3.3.), the amount of copper recovered with EDTA was initially high and decreased with time. The activity of plants could have been responsible for the increase in availability of soil copper during the pot trial but not the initial low availability.

Amounts of soil copper extractable with $CaCl_2$ before the trial were similar in all the soils except those treated with both $CuSO_4$ and the higher rate of either manganese chelate. The high values obtained may be the result of adsorption sites being occupied by Mn, since MnEDTA has a very low stability in soil (Norvell and Lindsay, 1969).

3.8.2.2. Plant yields (table 3.30.)

Although the soil used was very deficient in copper, addition of $CuSO_4$ on its own gave only small non-significant increases in barley yield. The higher rate of CuEDTA gave a small significant yield increase. At the first cut of clover, all copper treatments, apart from the surface applied $CuSO_4$ and the lower rate of CuEDTA, gave significant yield increases. The response was less with the second cut, with only three treatments giving significant yield increases, while the third cut gave no significant responses.

There was a significant yield response by ryegrass to both high and low levels of CuEDTA at the first cut but not to any other

Table 3.30.

Pot trial 2 : Plant yields

Crop	Treatment	Vegetation yields (g pot^{-1})					
		1st cut		2nd cut		3rd cut	
Barley (straw)	Control	7.1		-		-	
	$CuSO_4$	7.7					
	$MnSO_4$	6.9					
	$CuSO_4$ + $MnSO_4$	8.6	**				
	$CuSO_4$ + MnEDTA(low)	8.4	**				
	$CuSO_4$ + MnEDTA(high)	8.2	*				
	$MnSO_4$ Foliar	6.8					
	$CuSO_4$ + $MnSO_4$ Foliar	7.4					
	CuEDTA(low) + $MnSO_4$ Foliar	7.4					
	CuEDTA(high) + $MnSO_4$ Foliar	8.0	*				
	$CuSO_4$ + MnDTPA(low)	8.4	**				
	$CuSO_4$ + MnDTPA(high)	8.6	**				
	Treatment standard error	±0.4					
Clover	Control	7.0		11.9		6.2	
	$CuSO_4$	9.3	**	12.6		6.1	
	$CoSO_4$	7.8		13.1	*	6.1	
	$CuSO_4$ + $CoSO_4$	8.6	*	13.2	*	6.9	
	$CuSO_4$ + CoEDTA(low)	9.0	**	13.4	*	7.1	
	$CuSO_4$ + CoEDTA(high)	8.7	*	12.8		6.0	
	CuEDTA(low)	8.4		12.5		6.0	
	CuEDTA(high)	8.7	*	12.7		6.3	
	$CuSO_4$ surface	6.8		12.4		5.9	
	$CuSO_4$ surface after 1st cut	7.0		13.0		6.5	
	Treatment standard error	±0.5		±0.4		±0.3	
Ryegrass	Control	5.2		11.9		5.2	
	$CuSO_4$	5.4		12.6		7.3	
	$CoSO_4$	5.8		13.1	*	5.8	
	$CuSO_4$ + $CoSO_4$	6.0		13.2	*	9.1	
	$CuSO_4$ + CoEDTA(low)	5.9		13.3	*	6.9	
	$CuSO_4$ + CoEDTA(high)	5.6		12.8		8.1	
	CuEDTA(low)	6.6	**	12.5		8.4	
	CuEDTA(high)	6.5	**	12.7		7.7	
	$CuSO_4$ surface	5.6		12.5		6.6	
	$CuSO_4$ surface after 1st cut	5.1		13.0		6.1	
	Treatment standard error	±0.3		±0.4		±1.1	

treatment. The pattern was not repeated with subsequent cuts. It corresponds with the high recovery of CuEDTA from soils, immediately after addition, by extraction with EDTA. At the second cut, three treatments gave small significant responses similar to those obtained with clover. No treatments gave significant yield responses at the third cut.

The addition of $MnSO_4$ or the higher rate of MnDTPA with copper resulted in barley yields significantly higher than those from soils treated with $CuSO_4$ alone. Cobalt applications, however, had no significant effects on the yields of clover or ryegrass.

The observed differences in yields between treatments, which decreased with time, are likely to have been due to variations in the development of plant root systems. Thus, once the plants had established a good root system, the yield responses disappeared and, presumably, similar responses on this soil in the field would be short-lived.

3.8.2.3. Vegetation copper concentrations (table 3.31.)

Addition of copper to the soil substantially increased the copper concentration of barley straw, by about 100% in some cases. The treatments involving $CuSO_4$ and $MnSO_4$ or the high rate of MnDTPA (but not the high rate of MnEDTA), significantly increased the vegetation copper concentration above the level resulting from treatment with $CuSO_4$ alone. However, also in the presence of $CuSO_4$, the effect of the low rate of MnDTPA was significantly less than that of $MnSO_4$.

Large increases in the copper concentrations of clover at all three cuts resulted from mixing copper sulphate into the soil on its own, or in combination with cobalt, but no interaction occurred

Table 3.31.

Pot trial 2 : Vegetation copper concentrations

Crop	Treatment	Vegetation copper content ($\mu g\ g^{-1}$ DM)		
		1st cut	2nd cut	3rd cut
Barley (straw)	Control	1.9	-	-
	$CuSO_4$	3.3 ***		
	$MnSO_4$	2.2		
	$CuSO_4$ + $MnSO_4$	4.0 ***		
	$CuSO_4$ + MnEDTA(low)	3.6 ***		
	$CuSO_4$ + MnEDTA(high)	3.4 ***		
	$MnSO_4$(Foliar)	1.9		
	$CuSO_4$ + $MnSO_4$(Foliar)	3.1 ***		
	CuEDTA(low) + $MnSO_4$(Foliar)	2.3		
	CuEDTA(high) + $MnSO_4$(Foliar)	3.1 ***		
	$CuSO_4$ + MnDTPA(low)	3.2 ***		
	$CuSO_4$ + MnDTPA(high)	4.2 ***		
	Treatment standard error	±0.3		
Clover	Control	2.7	3.1	5.0
	$CuSO_4$	6.1 ***	8.2 ***	11.6 ***
	$CoSO_4$	3.0	4.2 *	5.9
	$CuSO_4$ + $CoSO_4$	5.7 ***	8.1 ***	10.9 ***
	$CuSO_4$ + CoEDTA(low)	5.6 ***	7.5 ***	10.1 ***
	$CuSO_4$ + CoEDTA(high)	5.5 ***	7.6 ***	9.5 ***
	CuEDTA(low)	3.4 *	4.8 **	6.1
	CuEDTA(high)	6.6 ***	7.7 ***	9.7 ***
	$CuSO_4$ surface	3.0	2.7	7.1 *
	$CuSO_4$ surface after 1st cut	±3.1	±3.6	±6.5
	Treatment standard error	±0.2	±0.3	±0.5
Ryegrass	Control	2.9	2.2	3.3
	$CuSO_4$	3.6 *	3.6 *	4.3 *
	$CoSO_4$	3.0	4.5 **	3.6
	$CuSO_4$ + $CoSO_4$	4.1 ***	4.3 **	5.0 ***
	$CuSO_4$ + CoEDTA(low)	4.2 ***	4.9 ***	4.4 *
	$CuSO_4$ + CoEDTA(high)	4.1 ***	4.0 ***	4.7 **
	CuEDTA(low)	3.0	3.5	3.9
	CuEDTA(high)	3.4	4.2 **	4.1
	$CuSO_4$ surface	2.6	2.5	3.5
	$CuSO_4$ surface after 1st cut	±2.7	±3.5	±3.9
	Treatment standard error	±0.2	±1.2	±0.3

between the two to influence copper uptake by the plants. As expected, after the findings of Reith and Mitchell (1964), ryegrass responded less readily to copper applications, although significant increases were obtained at all three cuts from all treatments involving the mixing-in of copper sulphate. The application of cobalt caused small, non-significant, increases in the copper concentration in ryegrass.

The high rate of CuEDTA produced highly significant increases in the copper concentration of barley and of all three cuts of clover but was no better than $CuSO_4$ in this respect. With ryegrass it only produced a significant increase in copper concentration at the second cut. The lower rate of CuEDTA produced smaller increases in copper concentration than the higher rate, which were significant only with clover and then only at the first two cuts. The results obtained indicate that there is no advantage, as far as the first crop on the treated soil is concerned, in applying CuEDTA instead of the considerably cheaper $CuSO_4$. The proportion of the applied copper available to plants appears to be similar in the two compounds. Thus, the application of CuEDTA at a lower rate, while reducing the cost of material, is of doubtful value.

Other studies, on both mineral and organic soils, have also shown organically complexed copper to be no more available than inorganic sources containing the same amount of copper (Kretchmer and Forsee, 1954; Younts, 1964; Gilkes and Lim-Nunez, 1979), although contrary results have been obtained (Beringer, 1963). The situation may well be different for calcareous soils, on which it has been demonstrated that CuEDTA is more effective than inorganic forms in

increasing copper uptake by bush beans (Wallace and Mueller, 1973), although under such conditions CuEDTA may be more easily lost by leaching (Korkman and Virta, 1979). On saline-alkaline soils, however, CuEDTA has been found to be the most appropriate form of copper supplement (Nabharan et al, 1977).

One possible reason for plants responding similarly to different forms of copper is that, when metal chelates such as CuEDTA come into contact with soil, the metal ions may be displaced from the chelate by other ions present in the soil at higher concentrations, such as ferric ions in acid soils (Norvell and Lindsay, 1969).

Applications of copper to the soil surface, made to pots containing ryegrass and clover, produced increases in vegetation copper concentration which were negligible in comparison to those obtained when copper was incorporated into the soil (figure 3.19.). Copper applied in such a way does not penetrate deeply into the soil, even when applied in a large volume of water (Beckwith, 1963) and is thus inaccessible to most of the plant root mass, since the initial adsorption apparently restricts subsequent mobility.

The effectiveness of a copper application mixed into the soil was also demonstrated by analysis of herbage samples from a field site (table 3.33.). Before the present pasture was sown, copper was worked into the topsoil of part of the field. Eight years later, herbage copper levels in the treated areas were still considerably enhanced. As in the greenhouse trial, clover gave a greater response than the grasses.

3.8.2.4. Vegetation manganese and iron concentrations (table 3.32.)

None of the soil treatments affected the manganese concentration

Figure 3.19.

Surface v. incorporated application of copper

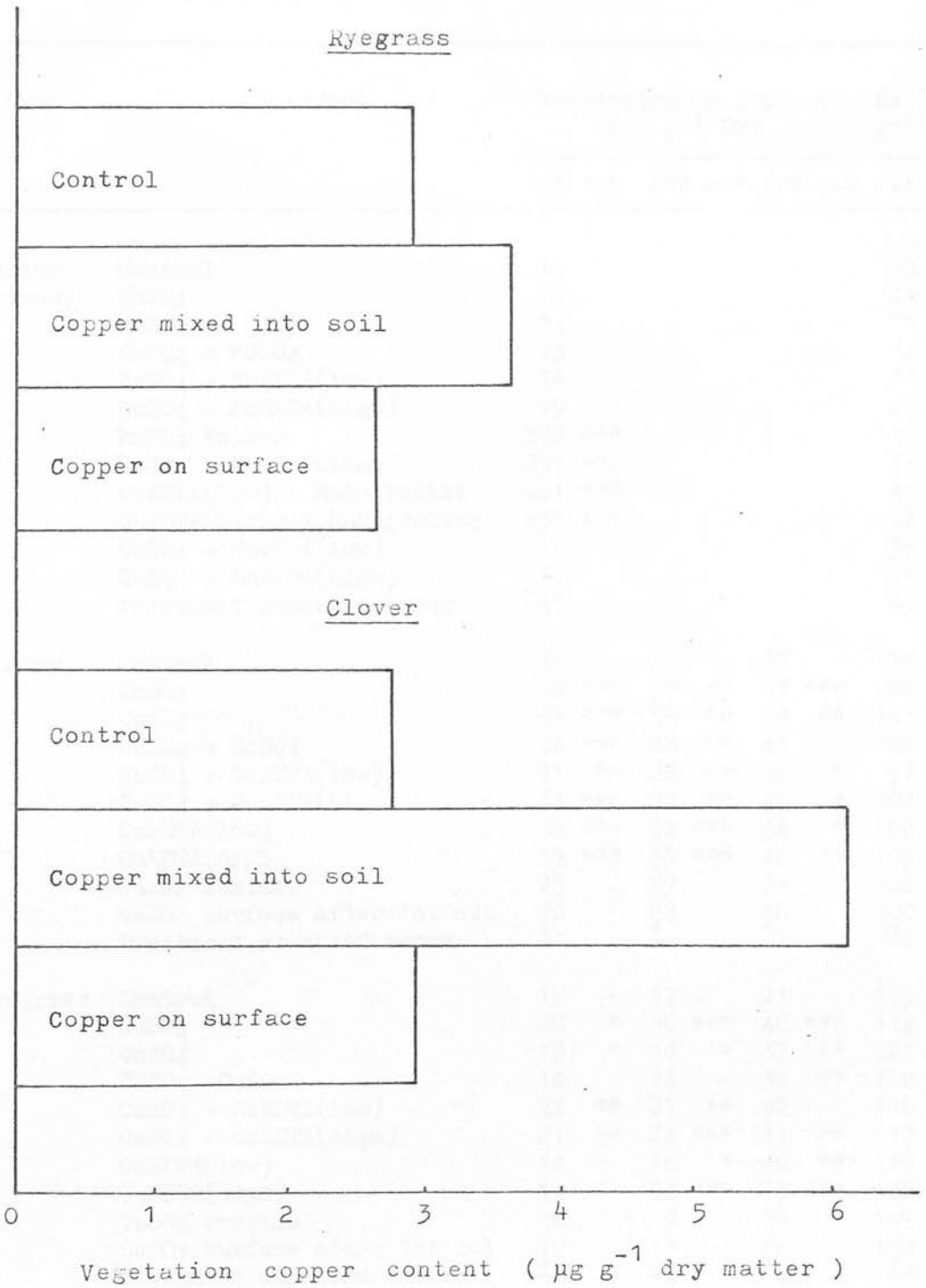


Table 3.32.

Pot trial 2 : Vegetation manganese and iron concentrations

Crop	Treatment	Vegetation Mn content (μg g^{-1} DM)						Fe (μg g^{-1} DM)	
		1st cut		2nd cut		3rd cut		1st cut	
Barley (straw)	Control	14						50	
	$CuSO_4$	10						41	
	$MnSO_4$	13						51	
	$CuSO_4$ + $MnSO_4$	13						44	
	$CuSO_4$ + MnEDTA(low)	16						36	
	$CuSO_4$ + MnEDTA(high)	19						41	
	$MnSO_4$ Foliar	286	***					45	
	$CuSO_4$ + MnSO Foliar	257	***					38	
	CuEDTA(low) + $MnSO_4$Foliar	261	***					41	
	CuEDTA(high) + $MnSO_4$Foliar	255	***					37	
	$CuSO_4$ + MnDTPA(low)	13						36	*
	$CuSO_4$ + MnDTPA(high)	17						57	
	Treatment standard error	±17						±5	
Clover	Control	26		21		38		114	
	$CuSO_4$	38	***	36	**	53	***	106	
	$CoSO_4$	32	***	29	**	49	**	103	
	$CuSO_4$ + $CoSO_4$	34	***	28	**	41		108	
	$CuSO_4$ + CoEDTA(low)	31	**	28	**	46	*	97	
	$CuSO_4$ + CoEDTA(high)	33	***	27	**	46	*	107	
	CuEDTA(low)	38	***	32	***	44	*	108	
	CuEDTA(high)	39	***	35	***	48	**	109	
	$CuSO_4$ surface	23		19		34		108	
	$CuSO_4$ surface after 1st cut	26		22		40		100	
	Treatment standard error	±1		±2		±2		±6	
Ryegrass	Control	12		12		21		135	
	$CuSO_4$	20	*	30	***	48	***	112	*
	$CoSO_4$	19	*	18	*	37	**	125	
	$CuSO_4$ +$CoSO_4$	14		16		38	**	120	
	$CuSO_4$ + CoEDTA(low)	22	**	21	**	32		110	
	$CuSO_4$ + CoEDTA(high)	21	**	24	***	41	**	117	**
	CuEDTA(low)	14		18	*	40	**	119	*
	CuEDTA(high)	13		24	***	53	***	88	**
	$CuSO_4$ surface	10		9		29		109	***
	$CuSO_4$ surface after 1st cut	12		13		24		129	**
	Treatment standard error	±2		±2		±4		±8	

Table 3.33.

Copper content of pasture at Rumbleton Rigg

Treatment	Copper ($\mu g\ g^{-1}$ dry matter) in vegetation sampled in June 1978		
	Mixed herbage	grass	Clover
No copper added	4.2	4.4	6.1
No copper added	3.9	4.9	5.4
11.2 kg $CuSO_4.5H_2O$ ha^{-1} in 1970	7.7	6.8	10.6
11.2 kg $CuSO_4.5H_2O$ ha^{-1} in 1975	5.1	5.2	9.1

of barley. High values were obtained where the plants had been sprayed with $MnSO_4$ but much of this would have been on the plant surfaces. However, most of the copper and cobalt treatments, with the exception of surface-applied copper, significantly enhanced the manganese concentrations of both the clover and ryegrass. This effect of copper on plant manganese concentrations has only previously been recorded in oat plants grown in peat (Dekock and Cheshire, 1968).

The iron concentration of the first cut of ryegrass was significantly depressed by some soil treatments. Clover was not affected but the iron concentration of one of the barley treatments was significantly depressed. Willis and Piland (1936) found that the application of copper sulphate to a peat soil decreased the uptake of iron by corn and, conversely, copper deficient oat plants have enhanced iron concentrations (Dekock and Cheshire, 1968). The interaction may be linked with the roles of both elements in electron transport within the plant.

3.8.3. Conclusions

Soil treatments increased plant yield only in the earlier cuts and the effect was thought to be transient.

The crops grown responded equally to CuEDTA and copper sulphate when both were used at the same rate of copper addition. A lower rate of CuEDTA, equivalent to the manufacturer's recommended rate, failed to increase the copper content of barley and ryegrass significantly. A slightly significant increase was obtained only with the earlier cuts of clover. In the situation studied, there was no advantage in using CuEDTA as a copper source.

The trial demonstrated the existence of interactions between copper and other trace elements. In particular, manganese additions to the soil in some cases resulted in higher copper levels in barley straw. With ryegrass and clover, manganese uptake was itself affected by copper additions. There was also evidence in the case of ryegrass of an interaction between copper and iron but interaction between copper and cobalt, two elements often applied to soil simultaneously in S.E. Scotland, was absent. This is important since, if interaction did take place, it could take the form of competition for uptake by plants.

Clover responded far more readily to soil application of copper, in terms of increased copper concentrations, than did ryegrass. Both plants responded poorly to copper applied to the soil surface instead of being mixed in. Thus top-dressings of copper compounds to permanent pasture are likely to be of little value.

3.9. Further studies of trace element interactions

In the field, the application of zinc has been found to enhance copper uptake by plants (F. P. Taylor, personal communication). Further investigation under more controlled conditions was indicated.

The enhancement of plant Mn content by soil copper treatment, observed in the previous trial, has seldom been recorded and could have been due to conditions peculiar to the trial.

A further pot trial was designed to look for interactions between copper, manganese and zinc which affect the uptake of these elements by ryegrass.

3.9.1. Method

The experiment was laid out in a randomised block design with one soil which received all combinations of presence or absence of single rates of the elements copper, manganese and zinc. Each treatment combination was replicated 3 times (3 blocks).

The same copper-deficient soil was used as in the previous pot trial. Treatment solutions were prepared in 200 ml distilled water to supply the appropriate elements at the rate of 2.5 $\mu g\ g^{-1}$ soil (table 3.34.). The soil was treated in the same way as in previous trials, except that 7" (178 mm) pots were used so that 9 kg soil was sufficient to fill the 3 pots comprising each treatment (2.75 kg soil pot^{-1}) and provide a separate sample for analysis. One pot of each treatment was placed at random in each of three blocks on benches in a greenhouse. After watering, 1g perennial ryegrass seed (*Lolium perenne* L. var. S2) was spread evenly over the surface of

Table 3.34.

Pot trial 3 : Treatments

All combinations of presence or absence of the following were included.

Compound	kg compound ha^{-1}	mg compound pot^{-1}	µg metal g^{-1} soil
$CuSO_4.5H_2O$	28.7	24.6	2.50
$MnCl_2.4H_2O$	26.2	22.5	2.50
$ZnSO_4.7H_2O$	32.0	27.5	2.50

each pot and covered with a little untreated soil. The trial was started in late October and artificial lighting and some heating were used. Distilled water was supplied to saucers under each pot and one surface application of a solution containing N, P and K (appendix 5) was made after 17 days. The plants were harvested after 52 days and analysed for copper, manganese and zinc.

3.9.2. Results and Discussion

An analysis of variance appropriate to the randomised block design of the trial was carried out on the plant properties determined (table 3.36.). The t-test was applied to the treatment means, using the overall standard error and its associated degrees of freedom.

There was no significant variation in plant yield.

Plant copper concentrations were significantly affected by soil treatment ($P<0.01$). Plants grown in soils treated with copper contained significantly more copper ($P<0.001$) than those grown in untreated soil. No other significant effects were found. Treatment of soil with zinc in addition to copper caused a small additional non-significant increase in plant copper concentration but when zinc was added alone the copper concentration of the vegetation was slightly decreased relative to the control. The latter observation corresponds with a report of copper deficiency in wheat as a result of applying zinc to a soil low in both copper and zinc (Loneragan, 1975).

With respect to plant manganese concentrations there was significant variation among both treatments and blocks, justifying

Table 3.35.

Pot trial 3 : Vegetation properties (mean values)

Treatment	Herbage yield (g pot^{-1})	Herbage contents (µg g^{-1} dry matter)		
		Cu	Mn	Zn
Control	3.0	2.91	10.26	256
Copper	2.9	6.30	9.70	274
Manganese	3.3	2.60	12.68	261
Copper + Manganese	3.1	5.63	10.97	278
Zinc	3.3	2.45	11.09	364
Copper + Zinc	2.8	7.06	8.42	301
Manganese + Zinc	3.2	2.38	11.76	307
Copper + Manganese + Zinc	3.2	6.76	10.23	320

Table 3.36.

Pot trial 3 : Analysis of variance

Property	Source of variation	Degrees of freedom	Sum of squares	Mean square	Variance ratio	Signif-icance
Foliage yield	Blocks	2	0.0014	0.0007	0.0190	
	Treatments	7	0.6841	0.0977	2.5564	
	Residual	14	0.5352	0.0382		
Copper content	Blocks	2	0.0212	0.0106	0.262	
	Treatments	7	93.08	13.30	32.86	***
	Residual	14	5.665	0.4046		
Manganese content	Blocks	2	4.807	2.403	4.037	*
	Treatments	7	35.64	5.092	8.553	***
	Residual	14	8.334	0.5952		
Zinc content	Blocks	2	3186.6	1593.3	0.8436	
	Treatments	7	26812.7	3830.4	2.0281	
	Residual	14	26440.1	1888.6		

the use of a randomised block design for the trial. Plant manganese concentrations was significantly enhanced by treatment of the soil with manganese ($P<0.01$). The effect was smaller when both manganese and zinc were added but was still significant ($P<0.05$). Treatment of the soil with copper depressed plant manganese concentration but the effect was significant when zinc also was incorporated in the soil ($P<0.05$). This trend is the opposite to that observed in the previous trial and corresponds with the observation of Beckwith (1963).

Although both trials were carried out on the same soil, the second one took place later in the year. Although heating was used, the soil temperature may have been lower. Loneragan (1975) observed that plant uptake of trace elements is sensitive to temperature. The copper and manganese concentrations in plants on untreated soil were similar in the two trials but the response to soil treatment varied. Treatment of soil with copper yielded higher plant copper concentrations in the second trial. Possibly the higher plant density in this trial resulted in an increased availability of copper to the plants, either as a result of improved soil-root contact or otherwise. Manganese concentrations in plants on treated soils were, however, higher in the first trial, in spite of the absence of added manganese. It would appear that environmental factors considerably modify the uptake of manganese by plants.

Neither treatments nor blocks varied significantly with respect to plant zinc concentrations so that all values might have been drawn from the same normal population. This would have been understandable if the soil had contained a high level of available zinc

but this was not so. A t-test indicated that treatment of soil with zinc significantly increased plant zinc concentration but, in the absence of an overall treatment effect, such a test is not statistically valid.

3.9.3. Conclusions

Soil treatments did not affect plant yield. Addition of copper significantly increased plant copper concentrations but the zinc treatment caused only small changes. Plant manganese concentrations were significantly enhanced by treatment of the soil with the element, a different effect to that obtained in the previous trial. Treatment of the soil with zinc had no significant effect on vegetation zinc concentrations. No evidence was found of interaction between manganese and zinc. The differences between the results of this and the previous trial were attributed to variations in environmental conditions.

3.10. General Summary

The reasons for studying copper in relation to its availability to plants, which were discussed in the introduction to the experimental work, indicated the importance of understanding the basic reactions in which copper is involved in soil.

Equilibria between copper in solution and that in the solid phase have received much attention in the past but techniques developed during the course of the current work, including the use of radioactive copper, have enabled the study of such equilibria at the low solution copper concentrations found in natural soils. In addition, the experiments have compared, under similar conditions, the reactions of copper with the main types of soil colloids. As a result of these studies it is considered that, in most soils, oxides and organic materials are likely to control the solution concentration of copper and thus its availability to plants. The direct influence of clay minerals may well be negligible. In short-term desorption studies only small amounts of the adsorbed copper were released into the solution phase but the resulting solution concentrations remained above the levels required for plant uptake. In addition, a considerable proportion of the adsorbed copper was shown to remain in isotopic equilibrium with the solution copper and thus be capable of ultimately desorbing into solution.

The relatively small effect of changes in major ion concentration on copper adsorption by soil colloids indicates that specific adsorption mechanisms are likely to be the most important reactions involved, with simple cation exchange playing a negligible role.

Within the pH range of most agricultural soils, variation in pH has only a small effect on solution copper concentrations. This is probably because only a small proportion of possible adsorption sites in a soil will be occupied by copper. In view of this and the fact that the equilibrium between solution and solid phase copper is heavily biased towards adsorption on the solid phase, changes in soil pH are unlikely to have much effect on copper availability to plants. This would appear to be borne out by observations in the field. Soil pH has a far smaller effect on copper uptake by plants than on the uptake of other trace elements, for instance cobalt and manganese.

Studies of adsorption and availability of copper using intact soils produced similar results to those obtained with individual soil materials.

Soluble organic matter was shown to be capable of complexing copper and thus elevating the overall concentration of copper in solution considerably above that resulting from the basic equilibrium between solid and solution phases. The extent of complexing in natural soil solution and the availability of the complexes to plants would be of considerable interest. However, preliminary work revealed existing techniques for examining complexing to be unreliable and any further work in this area would require a distinct improvement in methodology.

'Available' copper in soils is commonly determined by extraction with EDTA and the extractability of native and applied copper with this reagent has been examined in some detail. The amount of copper extracted has been observed to vary with environmental conditions

and does not always reflect changes in other copper fractions which, in some ways, may be considered as better indicators of copper availability, that is, readily soluble copper and isotopically exchangeable copper. The amount of copper extracted by EDTA was found to be greater than the amount of truly 'labile' copper in soil as determined by isotopic exchange. Although the fraction of soil copper extracted by EDTA has been used successfully for many years to predict copper deficiency in cereal crops, it was found to bear little relation to the uptake of copper by pasture crops. The level of EDTA-extractable copper accepted as being adequate for cereal crops is undoubtedly far too low, in many cases, to provide an adequate copper concentration in pasture herbage.

Pot trials demonstrated the existence of interactions, between copper and other trace elements, which affect the concentrations of these elements in plants, although the form and extent of the interactions appear to be affected by environmental conditions.

The size of the plant response to copper additions varied considerably from soil to soil. Further experiments of the type carried out are likely to enable predictions of copper requirements to be based on those soil properties which have been shown to affect the availability of copper. No advantage was apparent of applying copper to soil as CuEDTA rather than $CuSO_4$. Applied copper does, however, need to be worked into the soil for it to be readily accessible to plants.

The present studies have identified some of the factors which determine or modify the availability of soil copper to plants but further complexities have also been revealed. It is obvious that

further studies will be required to complete our understanding of the reactions of soil copper and its availability to plants.

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APPENDICES

Appendix 1 : Fractionation of copper in soils

a) Soluble and exchangeable copper

7g soil was shaken for 24 hours with 70 ml 0.05M $CaCl_2$ in a polypropylene centrifuge tube. After centrifuging at 6,800 rpm for 10 minutes, copper was extracted from 20 ml of the supernatant into 5 ml MIBK and determined by atomic absorption spectrophotometry, using electrothermal atomisation.

b) Weakly-bound specifically adsorbed copper

The same sample of soil was shaken with 70 ml 2.5% acetic acid for 24 hours. Centrifuging and analysis were as for the calcium chloride extracts.

c) Organically-bound copper

2g soil was shaken overnight with 200 ml 0.1M sodium pyrophosphate in a polypropylene centrifuge bottle. After centrifuging at 3,000 rpm for 20 minutes, 50 ml of the supernatant was pipetted into a beaker. On a hotplate the volume was reduced to about 25 ml and the contents evaporated to dryness with 5 ml aliquots of 30% H_2O_2 until the brown colouring of organic matter disappeared. The residue was dissolved with 10 ml N HCl and washed into a stoppered tube. Copper was extracted into 10 ml MIBK and determined by flame atomic absorption spectrophotometry.

d) Oxide-occluded copper

After extraction with pyrophosphate, the soil residue was washed into an evaporating basin and dried over a steam bath. 100 ml acid

oxalate solution (oxalic acid 0.1M, ammonium oxalate 0.175M) was added and an ultraviolet light placed overhead. The level in the basin was maintained with deionised distilled water. 2½ hours later, the sample was centrifuged at 3,000 rpm for 20 minutes. The solid was washed with another 25 ml acid oxalate, centrifuged again and the supernatants combined. Evaporation to dryness was followed by dry ashing at 450°C. The oxides were refluxed with 10 ml 3N HCl until they dissolved and the volume was made up to 20 ml in a volumetric flask with deionised distilled water. Copper was determined by flame atomic absorption, using standards in 1.5N HCl for calibration.

e) Residual copper

The soil residue from the oxalate extraction was returned to the evaporating basin, evaporated to dryness and then dry ashed at 450°C. After grinding to a fine powder with a pestle, 0.5g was weighed into a platinum crucible. 5 drops of water, 1 ml perchloric acid and 5 ml hydrofluoric acid were added and the crucible, partially covered, was placed on a sandbath at 220°C until dry. 2 ml water and 10 drops perchloric acid were then added and the crucible was returned to the sandbath. When it was dry, the residue was dissolved with 10 ml 2N HCl and the volume was made up to 20 ml in a volumetric flask, using deionised distilled water. Copper was determined in the same way as that in the oxalate extracts.

f) Total copper

This was determined in the same way as the residual copper fraction, using samples of soil which had been dry ashed at 450°C.

Appendix 2 : Extraction of solution copper into organic solvent

To an aliquot of aqueous sample in a 30 ml stoppered tube was added 1 ml of a 2% solution of Ammonium pyrrolidine dithiocarbamate (APDC) and a volume of Methyl isobutyl ketone (MIBK). The tube was then shaken by hand for two minutes and allowed to stand for two hours before analysis of the organic phase by atomic absorption spectrophotometry, using electrothermal atomisation.

Standard copper solutions were similarly extracted for the calibration of each batch of samples. For most determinations standards ranging from 0.01 to 0.09 $\mu g\ ml^{-1}$ were suitable. 5 ml of standard solution was extracted into 5 ml ketone but with the samples a concentration step of 10:1 was often obtained by shaking 20 ml of sample with 2 ml ketone.

The extraction was modified for acetic acid soil extracts, for which standards containing up to 2.5 $\mu g\ Cu\ ml^{-1}$ were necessary. With the pyrophosphate soil extracts, phase separation after shaking was often poor. After demonstrating that they did alter the copper concentration, Whatman phase separation papers were used to isolate the organic phase. Standards containing the appropriate high level of pyrophosphate were prepared for the determination of these extracts.

Appendix 3 : Soils used in incubation and pot experiments

Sample used in incub.	Sample used in pot expt.	Source	Grid reference	Soil series	Textural class
31	1	Colmsliehill	NT 514421	Ettrick	Clay loam
33	2	West Mains, Lauder (Highcross hill)	NT 557475	Lauder	Loam
35	3	Raescleugh	NT 613509	Linhope	Loam
45	4	Eastfield of Lempitlaw	NT 796333	Sourhope	Sandy loam
49	5	East Nesbit	NT 679265	Hobkirk	Sandy loam
*6	6	Mickle Harelaw-Harestane field	NT 661480	Hobkirk/ Eckford	Loamy sand

* also used for single-soil pot experiments.

Although only six sites are involved there are eleven different soils. Samples 31 - 49 are aggregated cores from the whole area of each field. Further work required larger samples which were collected, two years later, from a limited area of each field. Mechanical analysis indicated considerable differences between the two samples from each field, presumably due to variation within the field. The soils concerned were thus considered separately.

Soil 6 was obtained initially in sufficient quantity for all experiments.

Appendix 4

Change with time in

$CaCl_2$-extractable

soil copper

and isotopically

exchangeable copper

Soil	Treatment	$CaCl_2$-extractable copper ($\mu g\ g^{-1}$ soil)			Activity remaining in solution after equilibrating copper-64 with soil (%)			Isotopically exchangeable copper ($\mu g\ g^{-1}$)		
		6wks	12wks	36wks	6wks	12wks	36wks	6wks	12wks	36wks
31	dry	0.033	0.019	0.021	2.64	1.90	2.29	1.25	0.85	0.93
	incubated	0.028	0.021	0.030	2.80	2.12	2.03	1.05	0.97	1.84
	dry + Cu	0.051	0.063	0.049	2.22	1.78	1.85	2.29	3.29	2.65
	incub + Cu	0.057	0.045	0.053	1.65	1.16	1.23	3.49	1.92	4.41
33	dry	0.029	0.019	0.024	3.09	2.37	2.56	0.95	0.82	0.93
	incubated	0.022	0.020	0.029	2.07	1.60	1.42	1.15	1.31	2.09
	dry + Cu	0.071	0.067	0.062	2.20	1.69	1.70	3.19	3.95	3.61
	incub + Cu	0.064	0.044	0.059	2.53	1.78	1.58	2.53	2.59	3.80
35	dry	0.021	0.014	0.021	3.22	2.34	2.65	0.67	0.61	0.78
	incubated	0.024	0.011	0.018	2.31	1.55	1.34	1.06	0.78	1.35
	dry + Cu	0.047	0.063	0.049	1.88	1.65	1.79	2.46	3.82	2.75
	incub + Cu	0.043	0.033	0.051	1.69	1.24	1.10	2.72	1.91	4.73
45	dry	0.025	0.019	0.033	2.43	2.94	3.26	1.04	0.66	1.03
	incubated	0.031	0.060	0.028	1.85	1.69	2.36	1.71	3.83	1.41
	dry + Cu	0.058	0.054	0.065	1.64	1.83	2.42	3.50	2.93	2.67
	incub + Cu	0.055	0.085	0.066	1.45	1.31	1.84	3.87	6.54	2.93
49	dry	0.025	0.023	0.028	3.96	4.69	4.83	0.55	0.49	0.58
	incubated	0.026	0.030	0.025	3.14	3.50	4.74	0.88	0.84	0.55
	dry + Cu	0.056	0.074	0.089	2.41	2.79	3.13	2.32	2.63	2.81
	incub + Cu	0.085	0.083	0.073	2.19	2.18	3.71	3.93	3.78	1.98
6	dry	0.019	0.044	0.021	2.90	3.68	4.37	0.66	1.18	0.47
	incubated	0.023	0.033	0.032	2.74	3.13	3.61	0.85	1.16	1.77
	dry + Cu	0.065	0.059	0.091	1.53	1.91	2.47	4.25	3.09	3.67
	incub + Cu	0.045	0.071	0.038	1.54	1.70	3.20	2.94	4.26	1.26

Appendix 5 : Composition of nutrient solutions used in pot trials

Trial	Crop	Element	Rate (kg ha^{-1})	Amount supplied per pot and compound used
Chelate	Barley	N	60	368 mg NH_4NO_3
	"	P	13	122 mg KH_2PO_4
	"	K	21	122 mg KH_2PO_4 plus 41 mg K_2SO_4
Chelate & soil response	Ryegrass and clover	N	100	613 mg NH_4NO_3
	" " "	P	22	204 mg KH_2PO_4
	" " "	K	34	204 mg KH_2PO_4 plus 68 mg K_2SO_4
Interaction	Clover	N	125	306 mg NH_4NO_3
	"	P	27.5	102 mg KH_2PO_4
	"	K	42.5	102 mg KH_2PO_4 plus 34 mg K_2SO_4